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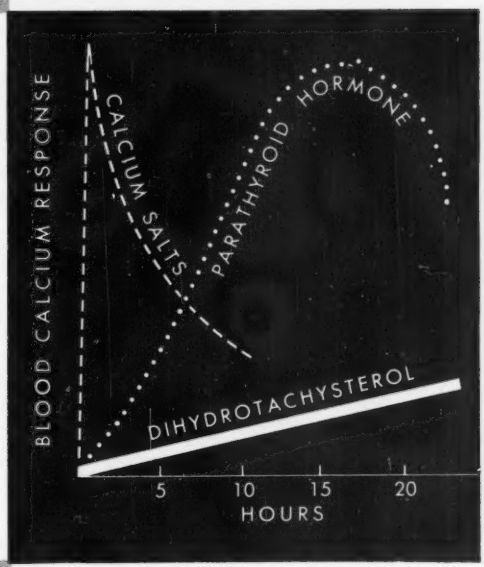

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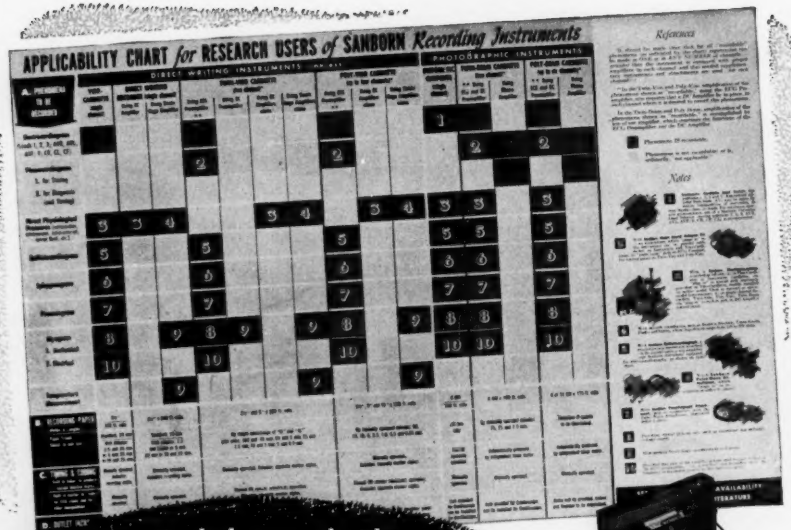

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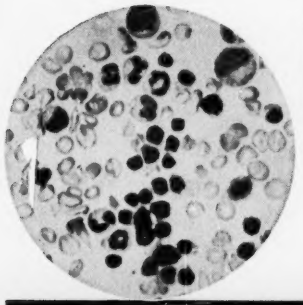

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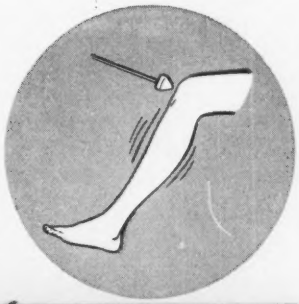

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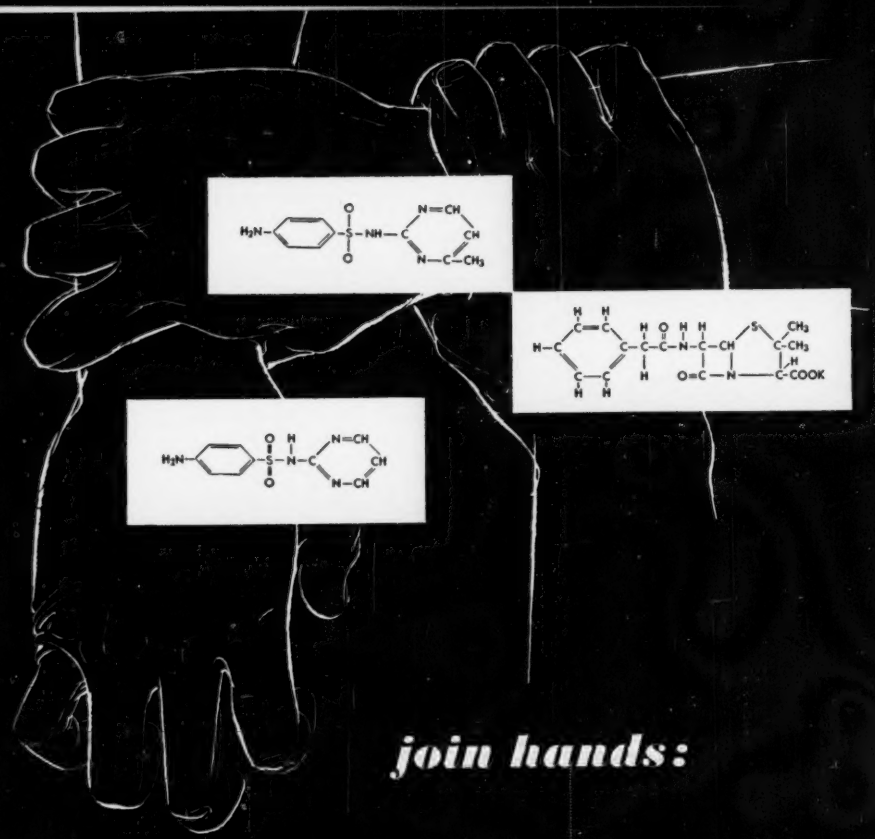

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THE EFFECTS OF TWENTY DEGREE HEAD-UP TILT UPON THE CEREBRAL CIRCULATION OF PATIENTS WITH ARTERIAL HYPERTENSION BEFORE AND AFTER SYMPATHECTOMY [1]

By JOSEPH H. HAFKENSCHIEL, CHARLES W. CRUMPTON,[2] HENRY A. SHENKIN, JOHN H. MOYER,[3] HAROLD A. ZINTEL, HERBERT WENDEL,[4] AND WILLIAM A. JEFFERS, WITH THE TECHNICAL ASSISTANCE OF SALLY CONLIN HARNED, NELLY J. KEFFER, AND GERALDINE CROFT

(From the Edward B. Robinette Foundation, Medical Clinic, Hospital of the University of Pennsylvania, the Graduate Hospital, the Department of Pharmacology, and the Harrison Department of Research Surgery of the School of Medicine of the University of Pennsylvania, Philadelphia, Pa.)

(Submitted for publication October 5, 1950; accepted May 23, 1951)

The present study was undertaken in order to measure the effect of a slight elevation of the head and shoulders upon the cerebral circulation of hypertensive patients following sympathectomy. Twenty degree tilt was chosen because it could be tolerated for 20 minutes, as early as two weeks after the Smithwick sympathectomy.

Shenkin and coworkers in 1948 reported that 20° head-up tilt did not alter the cerebral blood flow, cerebral oxygen consumption, and oxygen content of the internal jugular blood in five normal subjects (1). They also reported that, following thoracolumbar sympathectomy, cerebral oxygen uptake and blood flow in hypertensive patients while in the supine position did not differ from the preoperative level (2).

We have measured mean arterial pressure, blood flow, and oxygen uptake of the brain, before and after tilt in two groups of patients: (*a*) 15 patients who had been subjected to bilateral thoracolumbar (D6-L3) sympathectomy, and (*b*) 18 patients with essential hypertension.

METHODS

Patients with essential hypertension were selected from the medical and surgical wards of the Hospital of the University of Pennsylvania and the Graduate Hospital of the University of Pennsylvania. Preoperatively all had moderate to marked hypertension with retinopathy [5] grades II to IV (3), fluoroscopic and electrocardiographic evidences of left ventricular hypertrophy, and moderate impairment of renal function as measured by PSP excretion and urea clearance tests. At the time of the tilt study no patient showed either elevation of the blood urea nitrogen above 20 mgm. % or signs of congestive heart failure.

The patients studied after sympathectomy were selected at random without regard to the severity of the postoperative postural hypotension. All were ambulatory. Sympathectomies had been completed from two weeks to seven years prior to testing. Ten of the 17 tilts following sympathectomy were made in the 12 months immediately after operation, and of these ten, eight were studied within six months after operation.

Control measurements of cerebral blood flow, oxygen consumption, and mean femoral arterial pressure (4) were made in the supine position in a room kept as cool as was consistent with the patient's comfort. Accurate control of the environmental temperature was not possible. The patient was then tilted to a 20° head-up position. After three minutes in this position, simultaneously drawn samples of blood from the femoral artery and the internal jugular vein were obtained for analyses of oxygen and carbon dioxide content and blood pH as previously reported (5). When the patient had been in the head-up position for 20 minutes the second cerebral blood flow and oxygen consumption measurement was made. The pressure in the femoral artery during tilt and the vertical distance from the position of the needle to the level of the patients' eyes were recorded. The cerebral arterial pressure was then calculated as reported by Scheinberg and Stead (6). The jugular venous pressure was then subtracted from the calculated cerebral arterial pressure to give "effective" cerebral arterial pressure. Ve-

[1] This study was supported in part by research grants from the National Heart Institute, U. S. Public Health Service, the Life Insurance Medical Research Fund, Sandoz Pharmaceuticals, Irwin, Neisler and Company, and the Squibb Institute for Medical Research.

[2] Present address: University of Wisconsin, School of Medicine, Madison, Wis.

[3] Present address: Department of Medicine, Baylor University College of Medicine, Houston, Texas.

[4] Present address: Berlin—Zehlendorf, Irmgardstr., 45, U. S. Sector, Berlin, Germany.

[5] These examinations were made by Dr. Irving H. Leopold, Department of Ophthalmology, Hospital of the University of Pennsylvania.

nous pressure was measured (at the level of the internal jugular bulb) both while in the supine position and at the end of the tilt period while still semi-erect.

RESULTS

Table I contains the data on each patient. Also included are values previously reported for the normotensives. The percentage change and the significant differences from the supine value in each group are given in Table II. There were no highly significant differences between the mean differences of the three groups in any of the variables.

1. Mean arterial pressure

The "effective" cerebral mean arterial pressure was decreased by tilt in both groups of patients.

Eight patients tilted within six months or less after sympathectomy showed a highly significant change only in "effective" cerebral arterial pres-

TABLE I

Effects of 20° head-up tilt on cerebral circulation and oxygen metabolism

Patient group	Retinopathy grade	"Effective" cerebral mean arterial pressure, *mm. Hg**			Oxygen content internal jugular vein, *vol.%*			Arteriovenous oxygen difference, *vol.%*			Cerebral oxygen uptake, *cc./100 G./min.*		Cerebral blood flow, *cc./100 G./min.*		Vascular resistance†	
		S	3′	20′	S	3′	20′	S	3′	20′	S	20′	S	20′	S	20′
Essential hypertension																
H. H. 48 w m	III	144	118	116	9.9	10.5	10.0	4.5	4.3	4.4	3.5	3.2	77	73	1.9	1.6
D. L. 19 w f ‡	IV	141	128	112	12.0	11.5	11.0	4.7	5.4	5.6	3.3	4.0	70	71	2.0	1.6
W. R. 35 c m	II	114 (4.9)§	101	96 (4.1)§	11.1	10.7	9.8	5.4	6.0	6.9	3.1	3.6	58	52	2.0	1.8
W. M. 40 w m‡	IV	144 (9.2)	128	121	12.1		12.7	5.0		5.9	3.1	3.7	62	63	2.3	1.9
A. R. 33 w f ‡	II	130		126	9.7		9.2	8.8		9.9	4.0	4.1	45	41	2.9	3.1
A. S. 50 w m	III	127		113	10.5		8.9	6.3		8.3	3.1	3.7	49	45	2.6	2.5
N. M. 49 c f	III	147 (8.1)	113	104 (3.7)	10.8	10.5	9.5	5.9	5.6	6.2	2.9	3.7	49	60	2.8	1.7
H. M. 45 w f	IV	192 (2.9)	180	162 (2.2)	10.0	10.4	9.3	7.2	6.7	7.5	3.3	3.3	47	44	4.1	3.7
J. P. 43 w m	IV	151 (5.6)	114	128 (5.9)	9.6	8.3	8.5	5.2	6.4	6.4	2.3	2.9	48	45	3.1	2.8
E. K. 29 w f	II	129 (6.5)	110	111 (5.0)	12.7	12.3	12.2	5.4	5.6	5.6	5.0	5.1	93	91	1.4	1.2
W. W. 45 c m	II	151 (8.2)	130	138 (8.7)	11.9	11.8	10.9	5.7	6.8	6.4	4.0	4.2	70	65	2.2	2.1
L. B. 31 c f ‖	III	149 (6.2)	143	134	8.1	7.4	7.5	4.9	5.3	5.3	4.5	3.6	91	68	1.6	2.0
V. K. 41 w f	II	151 (8.1)	139	134	10.4	10.6	10.5	7.3	6.5	7.1	4.7	4.7	64	66	2.4	2.0
R. A. 45 c f ‖	II	149		98	12.4		11.3	6.0		6.3	4.1	4.5	68	72	2.2	1.4
S. B. 43 w f	II	159	124	123	11.3	10.1	10.2	6.2	6.8	6.7	3.6	3.8	58	56	2.7	2.2
W. B. 52 c m‖	III	156	166	111	8.4	9.9	7.8	4.9	5.3	5.5	3.3	3.4	67	61	2.3	1.8
A. T. 44 c f	III	151 (3.7)	132	130 (2.9)	10.4	8.7	9.2	6.3	7.5	7.6	2.6	3.3	42	43	3.6	3.0
J. D. 41 w m	II	147	140	137	11.8	11.4	11.4	8.2	7.6	7.9	2.7	2.8	33	36	4.5	3.8
Mean		146 (5.7)	131	122 (4.6)	10.7	10.2	10.0	6.0	6.1	6.6	3.5	3.8	61	58	2.6	2.2
Standard dev. indiv. diff.				13 (1.7)		0.7	0.6		0.8	0.6		0.4		7		0.3
Postsympathectomy (mos.)																
Y. J. 46 w f	6½	123		92	10.9	9.9	9.6	4.7	6.5	6.5	3.4	4.0	72	62	1.7	1.5
M. H. 33 w f	17	135 (9.1)	104	49 (6.0)	10.4	9.8	6.6	6.9	6.5	11.2	5.3	5.0	77	44	1.8	1.1
F. H. 33 w m	16	114	92	91	12.1	11.9	11.4	5.3	6.2	6.5	3.3	3.3	62	51	1.8	1.8
P. B. 46 w m	7	104 (5.9)	98	98	10.7	10.7	10.1	6.3	6.8	7.0	3.3	4.1	52	59	2.0	1.7
A. P. 32 w f	21	110 (9.1)	115	102	8.3	9.0	7.9	8.0	6.9	8.0	3.7	3.6	46	45	2.4	2.3
E. L. 43 w m	6	159 (8.1)	141	127 (7.4)	10.4	11.1	10.6	6.3	8.5	6.3	2.5	2.8	40	45	4.0	2.8
S. C. 33 c f	3 ¶	168 (7.5)	151	133	8.5	8.9	8.4	8.6	7.5	8.3	3.4	3.8	39	46	4.3	2.9
D. L. 19 w f	3 ¶	125 (9.9)	79	63 (8.8)	10.9	9.7	9.8	4.5	5.9	5.9	2.3	4.1	50	69	2.5	0.9
K. G. 40 w f	6	133 (4.9)	120	110 (4.6)	10.4	9.5	9.6	5.1	5.5	6.1	4.2	4.3	82	71	1.6	1.5
W. M. 40 w m	3½¶	92 (7.8)	83	83 (7.4)	12.4	11.2	11.2	5.2	6.4	7.0	3.8	3.9	73	55	1.3	1.5
F. K. 48 w f	3 ¶	101 (7.1)	100	60 (6.8)	9.1	9.6	8.6	6.4	5.5	6.9	4.3	4.8	67	69	1.5	0.9
M. D. 36 c f	7½	162 (8.5)	135	101 (7.7)	12.3	10.9	10.5	6.6	7.2	8.1	3.4	3.6	51	44	3.2	2.3
E. W. 49 w f	60	146 (5.5)	127	116 (3.3)	10.5	8.2	7.6	7.1	8.8	10.1	2.7	3.2	38	32	3.8	3.6
J. M. 28 w m	84	163 (7.7)	153	150 (7.5)	10.7	10.2	11.9	6.7	8.6	7.3	5.4	4.2	81	57	2.0	2.6
A. R. 33 w f	½	108		77	8.4		8.7	6.9		6.6	3.5	3.0	51	45	2.1	1.7
	14	94	77	70	8.7	7.4	7.8	6.1	8.0	7.6	3.9	3.9	64	52	1.5	1.3
	24	95	77	59	9.2	7.6	7.7	6.0	7.2	7.3	3.2	3.9	53	53	1.8	1.1
Mean		125 (7.6)	110	93 (6.6)	10.2	9.7	9.3	6.3	7.0	7.5	3.6	3.9	59	53	2.3	1.9
Standard dev. indiv. diff.				21 (1.1)		0.9	1.2		1.0	1.2		0.6		13		0.6
Normotensive patients previously reported (1) Mean		88		73	10.3		10.4	6.1		6.3	3.2	3.3	53	54	1.7	1.4
Standard dev. indiv. diff.				7			0.9			0.6		0.3		5		0.2

* S signifies supine position mean values.
3′ signifies mean values after patients had been three minutes in the 20° head-up position.
20′ signifies mean values after patients had been 20 minutes in the 20° head-up position.
† Signifies "effective" cerebral mean arterial pressure divided by cerebral blood flow.
‡ Signifies patients studied after sympathectomy.
§ Signifies jugular venous pressure that was subtracted from calculated cerebral pressure to give "effective" pressure.
‖ Signifies patient had symptoms after 12 minutes head-up requiring return to supine position.
¶ Signifies patient had entire right adrenal removed at time of second stage sympathectomy.

TABLE II

Comparison of the per cent changes in cerebral circulation and oxygen metabolism with 20° tilt

Patient group	Cerebral					
	"Effective" mean arterial pressure	Jugular oxygen content	Blood flow	Vascular resistance	Oxygen uptake	A-V O_2 difference
Essential hypertension, 18 pts., 18 observ'ns	−16*	−6*	−5	−15*	+9*	+10*
Postsympathectomy, 15 pts., 17 observ'ns	−26*	−9*	−11	−17*	+8	+19*
Normotensive, 5 pts., 5 observ'ns. Prev. reported (1)	−17*	+1	+2	−18	+3	+3

* Signifies statistically significant differences from the mean values in the supine position ($p < 0.01$).

sure. The 26% reduction was identical with that of the entire postsympathectomy group.

2. Cerebral venous blood oxygen content and brain blood flow

In both groups of patients the internal jugular blood oxygen concentration was reduced significantly from the recumbent value ($p < 0.01$). Comparable increases in the cerebral arteriovenous oxygen differences were observed. The increase in this oxygen difference after three minutes of tilt was significant in the patients studied after sympathectomy ($p < 0.02$), but was not in the patients with essential hypertension. The decreases in the oxygen content of cerebral venous blood after 20 minutes of tilt cannot be attributed to significant reductions in cerebral blood flow in either group (Table II).

3. Cerebral oxygen consumption

Although the cerebral arteriovenous oxygen difference increased only 10% in the hypertensives, the 9% increase in cerebral oxygen consumption after 20 minutes of tilt proved to be highly significant ($p < 0.01$). Cerebral oxygen consumption was not increased in the postsympathectomy group.

4. Cerebrovascular resistance

The cerebral blood flow of the patients after sympathectomy was decreased 11% with a 26% reduction in "effective" mean cerebral pressure. This can be explained by the fact that the cerebrovascular resistance decreased 17% from 2.3 to 1.9 units, indicating a highly significant relaxation of cerebral vessels during the tilt period. A comparable reduction in cerebrovascular resistance was found both in the hypertensive patients and in the normotensive subjects (1).

5. Untoward symptoms during tilt

It is noteworthy that no symptoms suggesting an inadequate cerebral circulation were encountered while the surgically treated patients were tilted. However, three patients with essential hypertension, L. B., R. A., and W. B. (Table I), having been immobile at least 12 minutes in the tilt position, suddenly developed sweating, nausea, weakness, bradycardia, marked drop in blood pressure and syncope. These were ameliorated by a return to the horizontal and the 20 minute observations in these patients were made there. R. A. and W. B. had reductions in pressure considerably greater than the mean of the group, indicating that the pressure had not returned to the pretilt level over a 15 minute period following the fainting episode. The other patient, L. B., had a 25% fall in blood flow (91 to 68) even though the pressure rose toward the control observation. There was an increase in resistance in this patient, without an appreciable increase in the cerebral arteriovenous oxygen difference. Each of these patients showed a decrease of 0.6 vol. % or more in the oxygen content of the internal jugular blood when compared with the pretilt concentration. It is of interest that L. B. had a transient left hemiplegia one month before. Some slight weakness of the left leg was present at the time of this study. The supine cerebral blood flow, cerebral arteriovenous oxygen difference, cerebral oxygen uptake, and

cerebrovascular resistance were not characteristic of essential hypertension (5).

6. Tilt studies before and at intervals after sympathectomy in the same patient

Postural hypotension of a moderate degree was observed with the tilt test over the two year period following sympathectomy in patient A. R. (Table I). Although this patient had only moderate hypertension preoperatively, she was troubled with severe headaches and had evidence of vascular damage of such a degree as to warrant sympathectomy. Following operation, she had complete relief from headache. The fundi are now normal. The heart size and electrocardiogram are now normal. Renal dysfunction has not progressed.

Tilt tests were made before operation, two weeks, 14 months, and two years after sympathectomy. The cerebral blood flow during the most recent tilt study was unchanged when the "effective" mean cerebral pressure was reduced 38%. These measurements indicate that the cerebral vessels in this patient are capable of considerable relaxation at this time, cerebrovascular resistance having decreased equally with the arterial pressure. There was, however, a 25% increase in cerebral arteriovenous oxygen difference and oxygen consumption as jugular blood oxygen content decreased 1.5 vol. % when compared with the supine observation. No unpleasant symptoms were observed during this tilt period. Two other patients were studied before as well as after sympathectomy (D. L. and W. M., Table I). Cerebral blood flow increased with tilt in one, but decreased in the other. Both of these patients have had a good clinical response for at least one year after operation.

COMMENT

These studies offer additional evidence that the high tone of cerebral vessels normally present in essential hypertension is capable of partial relaxation in response to a fall in cerebral arterial pressure induced by 20° tilt for 20 minutes. A comparable degree of relaxation, tending to counteract the decrease in cerebral blood flow which would otherwise occur, has been found in the group tilted after sympathectomy. The decrease in the resistance in the latter group during tilt appeared to augment the previously mentioned reduction in cerebrovascular resistance in the supine position after sympathectomy (2). Although the cerebral oxygen uptake of the sympathectomy group was unchanged by the 20° head-up position, there was a highly significant reduction in the oxygen content of the jugular blood in *both* groups. Kety and associates have observed a similar trend in a study of the cerebral circulatory response to the acute reduction in cerebral mean arterial pressure induced by differential spinal anesthesia (7). We interpret this as an indication that the increased tone of the cerebral vessels usually present in essential hypertension and persisting after sympathectomy could only be partly relaxed in response to the fall in pressure induced by this slight degree of tilt.

The cerebral arteriovenous oxygen difference was not increased by tilt as much in the patients with essential hypertension as in the patients tested after sympathectomy (Table II). Cerebral blood flow was reduced about the same amount. However, the 9% increase in cerebral oxygen uptake was highly significant ($p < 0.01$). This was not observed in the normotensive group. The measurements of this study offer us no explanation.

The symptoms experienced by hypertensive patients during tilt were similar to those observed during a sudden fall in arterial pressure from differential spinal sympathetic block (7). These data are further evidence suggesting that such symptoms were the result of cerebral anoxia associated with an inadequate circulation while in this position. A decrease in cerebral blood flow and an increase in the cerebral arteriovenous oxygen difference have been reported when normotensive subjects were tilted to a 65° head-up position and held there for ten minutes (6).

Our data give us no ready explanation for the difference in the symptomatic response of the two hypertensive groups to this slight degree of head-up tilt. It is probable that the absence of collapse symptoms in the postsympathectomy group was fortuitous.

The patient E. L. (Table I) was studied six months after sympathectomy. He had suffered a right hemiplegia one week after the second stage was completed. Although this patient had complete motor and sensory recovery within the next

week, some weakness of the right leg remained at the time of this tilt study. E. L. had been studied before operation and there appeared to be no essential difference in the supine data, published as part of a report of the effect of stellate block (8). However, the 16% increase in blood flow during tilt with no decrease in cerebral arteriovenous difference raised the question of shunts through damaged cerebral tissue. Also of interest is the fact that the patient suffered a right homonymous hemianopia six weeks after the tilt study. This gradually cleared within three months.

Kety (9) and Schmidt (10) (Table II) have recently summarized the evidence from investigations over the past four years bearing on the relationship between arterial hypertension and the marked increase in cerebral vascular resistance accompanying it. They suggest a humoral agent as the mediator of the increased cerebral vascular resistance as well as the uniform increase in vascular tone throughout the body. The results of the present study in which hypotension was induced in the cerebral arterial system under slight physiologic stress also show that the increased cerebral vascular resistance can be decreased when the "effective" arterial pressure is lowered. The failure of the cerebral vasculature to relax to the degree enabling the oxygen content of the internal jugular vein blood to remain unchanged might be considered as further evidence suggesting the presence of a circulating pressor substance.

That relaxation of the tone of cerebral blood vessels is possible in hypertension has been demonstrated by studies of the cerebral circulation after sympathectomy (2) and following intramuscular dihydroergocornine (11). Following the administration of this drug cerebral blood flow, cerebral arteriovenous oxygen difference, and oxygen content of the internal jugular vein were essentially unchanged when the arterial pressure was significantly reduced. These latter observations seem to answer in the affirmative the question: Can the elevated arterial pressure of patients with essential hypertension be lowered without untoward symptoms that might be related to a decrease in cerebral blood flow, cerebral oxygen uptake, and mean cerebral oxygen tension? [6]

[6] The authors recognize that venous oxygen content is a less adequate measure of cerebral oxygen homeostasis than venous oxygen saturation or tension (7).

SUMMARY

Measurements of cerebral blood flow, cerebral oxygen uptake and cerebral vascular resistance were made by the nitrous oxide method before and after 20° head-up tilt in patients with essential hypertension. A significant reduction in "effective" cerebral arterial pressure during the tilt was observed.

Cerebral blood flow was not reduced significantly during tilt in either group. Cerebral vascular resistance was reduced in both. Oxygen uptake of the brain increased when the patients with essential hypertension were tilted and was unchanged in the sympathectomy group. Symptoms of cerebral anoxia were not observed in these patients.

The oxygen content of the blood from the internal jugular vein in the two groups of patients was significantly reduced while semi-erect. This indicates that, although the cerebral vascular resistance was diminished, the increased tone was only partially relaxed.

From the data obtained we believe the following conclusions may be drawn:

1. The increased cerebral vascular resistance in essential hypertension can be partially relaxed when the arterial pressure is lowered by this slight degree of tilt.

2. Patients studied after sympathectomy showed the same degree of incomplete relaxation with tilt as the patients with essential hypertension, but did not experience cerebral anoxia.

3. Failure of the cerebral vasculature to relax completely might be considered as evidence suggesting the presence of significant amounts of circulating pressor substances even in the patients who have had a sympathectomy.

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ERRATUM

In the article entitled "The Measurement of C-reactive Protein in Human Sera. Comparison of the Clinical Tests on the Basis of a Quantitative Method," by Harrison F. Wood and Maclyn McCarty, in the June 1951 issue (pp. 616–622), the data giving the concentration of C-reactive protein in human sera were calculated without allowance for the twofold concentration employed in the final step of the quantitative method. Consequently, the values for the concentration of the protein in human sera found in Table IV and in the text should be divided by two to obtain the correct figures.

STUDIES OF SERUM CREATINE TOLERANCE. I. FINDINGS IN NORMAL MALES AND IN HYPERTHYROIDISM [1, 2]

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In 1929, Palmer, Carson, and Sloan (1) reported that treatment of exophthalmic goiter with Lugol's solution reduced or abolished the associated creatinuria. More recently Richardson and Shorr (2, 3) and Thorn (4) have shown that hyperthyroid individuals excrete a larger proportion of a given dose of creatine than do comparable normal individuals, and that this "reduced tolerance" is partially or totally reversible by effective therapy. The development by one of us of a practical method for the determination of creatine in serum (5) led Tierney and J. P. Peters to investigate the effects of exogenous creatine on serum levels in normal humans and in thyroid disease (6). Their findings revealed that the increased creatinuria reflected increased serum concentrations, and that the "critical" serum level for the excretion of creatine was approximately 0.6 mg. %.

Because of the differences in the mode of excretion of creatine by adult males and females, we have undertaken a study of serum creatine tolerances of men with hyperthyroidism and of others with clinical conditions simulating Graves' disease in order to further define the nature of sex differences in creatine metabolism. It also seemed important to determine whether the *increased* tolerance noted by Tierney and Peters in one male (6) was a characteristic of this disease in men.

MATERIALS AND METHODS

Thirteen "normal" subjects were selected from laboratory personnel, faculty members and resident physicians (Table I). Patients, most of whom were hospitalized, were selected in whom the diagnosis of hyperthyroidism was established or suspected. In addition, certain individuals who had non-toxic thyroid enlargement were tested. Ultimate diagnosis in each case was based on clinical observations, determination of serum precipitable iodine prior to therapy, and single or serial measurements of basal metabolic rate and serum cholesterol (Tables II and III). In most instances, serum protein and albumin were also determined in order to rule out hypoalbuminemia as a possible cause of confusion in the interpretation of serum iodine data (7), and in two patients,

TABLE I

Normal subjects

Patient	Sex	Age	Date*	Serum iodine γ %
W. B.	M	27	8/30/50	
K. G.	M	27	8/31/50	5.4
G. R.	M	28	9/ 1/50	
F. M.	M	29	9/15/50	5.5
L. N.	M	21	10/23/50	5.7
G. H.	M	25	11/ 8/50	4.7
C. C.	M	29	11/16/50	
M. S.	M	30	11/ 7/50	
M. L.	F	22	11/14/50	5.3
P. L.	F	22	1/18/51	3.9
D. B.	F	26	1/22/51	
E. C.	F	23	1/23/51	7.3
M. W.	F	27	1/23/51	5.5

* Refers to dates of creatine tolerances illustrated in Figure 1. Serum iodine was determined prior to tolerances in most instances.

serum "thyroxine" concentration (8) was also measured to verify the functional significance of artifactually elevated iodine values (9). The euthyroid state of a majority of the normal subjects had been verified by determination of serum precipitable iodine (8) in the course of other investigations of thyroid function (10).

Creatine tolerance tests were performed prior to therapy, ten days after the start of therapy, preoperatively and postoperatively whenever possible. The hospital patients were kept at bed rest during the tolerance periods, but the normal subjects and outpatients were permitted to indulge in limited ambulatory activity in the laboratory. The procedure followed was that of Tierney and Peters (6) with the following exceptions: (*a*) the inter-

[1] This investigation was supported in part by a research grant from the National Institutes of Health, Public Health Service.

[2] Sponsored by the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are a result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

vals for collection of specimens were varied for reasons discussed under Results below, (*b*) urine collections were omitted, since other data have demonstrated that in the absence of renal disease, the presence or absence of creatinuria is governed by serum creatine concentration (6, 11, 12), and (*c*) autoclaving of specimens for the analysis of total creatinine was carried out in 12 ml. volumetric flasks rather than in colorimeter tubes so that any evapora-

TABLE II

Thyrotoxicosis—clinical data

Patient Age No.	Date*	Figure ref.	Wt. (*Kg.*)	Pulse (*/min.*)	BMR (%)	Serum			Therapy				Clinical
						Chol. (*mg. %*)	Iodine (*γ %*)	Albumin (*gm. %*)	Date started	Lugol's (*drops /d*)	Propyl thiour. (*mg./d.*)	Operation date	
J. S. 49 No. 72609	8/22/49 9/ 6/49	2A 3A	60.1 62.8	76 76	+74 +12	174	13.1		8/26	30	300		Rheum. ht. disease, fibrillation, failure. Large thyroid, clinically toxic. Left hospital against advice.
H. N. 30 No. 78051	8/26/49 9/13/49 10/ 3/49 3/16/50	2A 3A 4A 4B	70.1 70.5 72.8 78.7	90 88 88 80	+85 +16 +29	171	12.3	 5.2	 9/1	 30 30	 300 300	 11/25	Clinical opinion divided. Majority for thyrotoxicosis. Path: diffuse colloid involution.
A. S. 29 No. 79501	11/ 2/49 11/17/49 12/ 5/49 4/27/50	2A 3A 4A 4B	64.6 68.7 75.5	120 120 108 90	+49 +44 +24 + 9	121 245	18.3	 5.1	11/3 11/14 12/3	 30	450 300 450	 1/27	Clinically classic.
B. J. 62 No. 76206	1/10/50 2/ 7/50 2/27/50 4/25/50	2A 3A 4A 4B	72.3 71.9 72.8 74.6	90 92 80	+34 +42 +40 + 7	222	25.3†		1/25		300 300	 4/14	Toxicity questioned clinically. Had been on Lugol's gtts. xv/d. for 4 mos. prior to to 1/1/50. Path: colloid adenoma and chronic thyroiditis; local.
R. R. 26 No. 80185	1/20/50 2/ 3/50 2/21/50 5/ 3/50	2A 3A 4A 4B	 59.6 62.3 73.3	116 96 110 88	+53 +60 +34 + 4	121 295	22.8	 5.7	 1/21 2/23	 	 400 600	 4/20	Clinically classic. Path: hyperplasia with involution.
F. B. 24 No. 76606	1/31/50 2/10/50 3/ 2/50 3/17/50	2A 3A 4A 4B	54.1 56.9 63.7 64.2	 84 80	+80 +68 +24 + 9	145	11.2	 5.1	 1/31 2/16	 15	 300 300	 3/8	Clinically classic.
F. G. 54 No. 82795	4/ 3/50 4/24/50 5/17/50	2A 3A 4B	62.2 63.1 64.0	92 84 80	+32 − 4	178 208	10.4	3.9	4/11	15		5/10	Clinically doubtful. Path: colloid and fetal adenomata.
S. M. 57 No. 77983	4/ 7/50	2A	60.4	72	− 4	269	8.6	4.5				5/3	Clinically non-toxic adenoma. No preoperative therapy. Path: multiple colloid adenomata.
C. B. 23 No. 78465	4/14/50	2A	68.2	100	+32	208	12.1	4.5					Clinically classic—left hospital without therapy.
L. McM. 55 No. 78493	5/25/50 6/ 9/50 6/29/50	2A 3A 4A	43.6 44.0 44.9	80 76 80	+26 +12 − 1	160 296	16.1	4.5	5/29 5/31 6/12		200 300 200		Classic "apathetic" thyrotoxicosis. Not operated. X-ray: decreased cardiac shadow on treatment.
W. G. 27 No. 80842	7/17/50 7/28/50 8/18/50 9/13/50	2A 3A 4A 4B	49.5 53.6 55.8 59.0	100 100 96 76	+58 +37 +12 − 4	186	17.9	4.6	 7/17 8/20	 	 300 400	 9/6	Clinically classic.
A. T. 29 No. 80940	7/17/50 7/31/50 8/21/50 9/20/50 10/18/50	2A 3A 4A 4A 4B	64.9 66.7 74.0 75.1	112 100 90 80 76	+49 + 9 +44 +16 + 9	315	16.2	4.6	7/21		300 300 300	1/10	Thyroidectomy for thyrotoxicosis 10 months previously. On small dosage of propyl thiouracil for 6 mos. prior to admission because of recurrence. Clinically classic.
J. H. 44 No. A2906	9/26/50 11/ 7/50	2A 4A	47.3 56.0	88 83	+63 +16	211	18.4		10/25	30	300		Clinically classic with exophthalmos. I^{131}–86% retention.
E. M. 54 No. A3095	10/17/50	2A	51.4	84	+36	200	14.1	4.0					Classic with exophthalmos and heart disease. Previous operation.

* Date on which creatine tolerance was performed. In most instances other data obtained within ±72 hrs. Serum for iodines drawn same day as tolerances.

† Previously on Lugol's solution. Serum thyroxine 10.05γ%.

tion losses in autoclaving could be made up quantitatively with distilled water prior to transfer for colorimetry. The increased creatinuria usually attributed to omission of a preliminary period of "creatine-free" diet is not sufficient to significantly affect the results of these studies (11, 13). Throughout this paper, the convention of reporting creatine values in terms of creatinine is followed.

RESULTS

Normal tolerance curves

Serum tolerances of eight young men (Table I) plus the two curves reported by Tierney and Peters (6) [3] are presented in Figure 1A. It is readily apparent that reducing the number of determinations to three and reducing the duration of the test from 180 to 135 minutes gives essentially the same spread of values as the four-point (fasting, 30, 60, and 180 minutes) routine previously employed (6). That this is, for practical purposes, also true in abnormal curves will be clear from the data which follow. The data represented in Figure 1B strongly suggest that this modification is also applicable in normal females. The noteworthy characteristics of the normal male curve are: (*a*) fasting levels at or below 0.6 mg. %, the level which approximates the minimum serum level associated with creatinuria, (*b*) a definite rise at the intermediate (30 to 60 minutes) interval with considerable variation in degree of this rise, and (*c*) a return at 135 or 180 minutes to serum concentrations of 1 mg. % or less. These details contrast with the normal females in whom fasting concentrations frequently exceed 0.6 mg. %, and whose final values are usually in excess of 1.00 mg. % (Figure 1B).

[3] With the permission of the authors and publishers, data from their paper have been used in Figures 1-3 in order that our curves may readily be compared with similar findings in females.

Untreated hyperthyroidism

In Figure 2A, it is apparent that the curves in these patients are quite different from the normal male group. Of the 14 cases represented only one value falls below the upper limit of the normal range at 135 minutes. Thus, the alterations seen in this group are similar to those previously reported in females ([6]and Figure 2B), although none reach the high concentrations seen in patient S. H. at 30 and 60 minutes. Laboratory and clinical data are summarized in Table II. In patients B. J. and F. G., the tolerance pattern confirmed other laboratory findings, particularly the iodine values, although originally clinical opinion was divided and final diagnostic decision had to be based on response to therapy. The curve which falls to the normal range represents a case in whom clinical opinion was also divided, despite a serum precipitable iodine concentration of 12.3 γ % (H. N.—Table II). Again, the ultimate diagnosis of hyperthyroidism was based on therapeutic re-

TABLE III

Miscellaneous conditions

Pt.	Age	No.	Date	Pulse	BMR (%)	Serum			Operation date	Clinical
						Chol. (mg. %)	Iodine (γ %)	Albumin (gm. %)		
H. W.	25	77070	2/23/50	78	+ 9	261	5.3	5.4		Clinically thyroiditis or non-toxic adenoma.
H. C.	54	72479	9/ 9/49	108	+42	228	55.2*			Exophthalmos. Clinically questionable.
			10/ 4/49	100	+31			5.0		Died of sarcoma. Path: normal thyroid.
E. L.	48	71927	8/25/49	76	− 9	231	3.8			Non-toxic adenoma.
J. C.	59	78362	5/ 5/50	90	+ 7	228	7.5			Non-toxic nodular goiter.
			6/ 9/50	80					6/2	Path: fetal adenomas and colloid adenomas.
E. D.	38	78980	5/ 4/50	75	− 6	333	4.4		5/8	Non-toxic adenoma. Path: colloid adenoma.
C. D.	72	75487	12/29/49	60	+18	145	8.9†			Thyroidectomy 1938. Cardiac failure.
			1/25/50	84	+ 4	163	7.1‡	4.6		On propyl thiouracil 12/31–2/13.
			2/15/50		+ 4	268				No clinical response to medication.
J. S.	22	OPD	10/ 7/50	65	− 3		5.1			Wt. loss, nervousness, sweating.

* Artifact due to "Priodax" cholecystography.
† Result questionable—? previous iodine therapy.
‡ Thyroxine—3.9γ%.

sponse. Patient S. M. was inadequately studied in that, unfortunately, complete follow-up was impossible. Thus although the creatine tolerance curve and serum iodine values were in the hyperthyroid range, all other evidence pointed to a nontoxic adenoma, and the patient was treated accordingly. However, on a postoperative visit in January, 1951 the patient was found to have gained ten pounds. If S. M. is considered to have been euthyroid, 12 of the 14 tolerances were in accord with the final diagnosis, one was falsely normal, and one was falsely hyperthyroid (H. N. and S. M., respectively). None of the curves in this series are of the flat type shown by the one hyperthyroid male previously reported ([6] and Figure 2A).

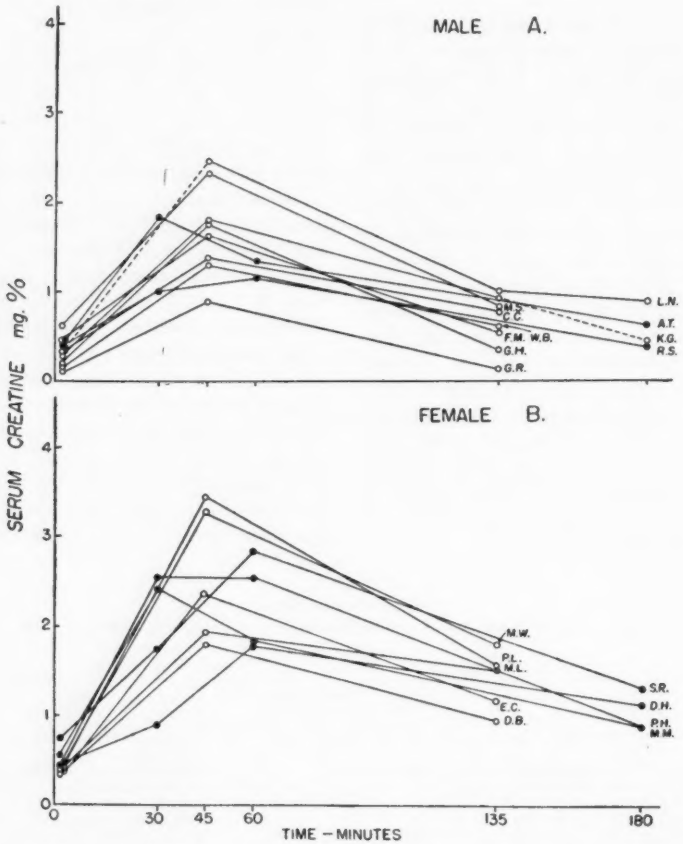

FIG. 1. NORMAL ADULTS

Open circles represent authors' cases, solid circles from Tierney and Peters (6). *A. Males.* In two cases, points connected to curves by a broken line were determined on a separate occasion, in one instance because of a laboratory accident, in the other because the 135 minute value was suspiciously high. *B. Females.* Comparison of the male and female groups at 135 minutes, using the "t" test, reveals a "p" value of 0.001, indicating that the sex difference is a real one. No such difference is demonstrable at 30, 45, or 60 minutes. The highest male fasting concentration is 0.60, approximately the "threshold" level for creatinuria.

Effects of treatment

Figure 3A demonstrates the prompt return of all but one of the tolerance curves to the normal area early in the course of medical treatment. Thus one case treated with Lugol's solution, seven with propyl thiouracil and two who received both drugs all display normal male patterns. Females, on the other hand (Figure 3B), have been shown to respond less completely when given Lugol's solution.

The curves in Figure 4A, representing later stages in the course of preoperative preparation, or of medical therapy in unoperated cases, indicate that the correction of the defect in creatine

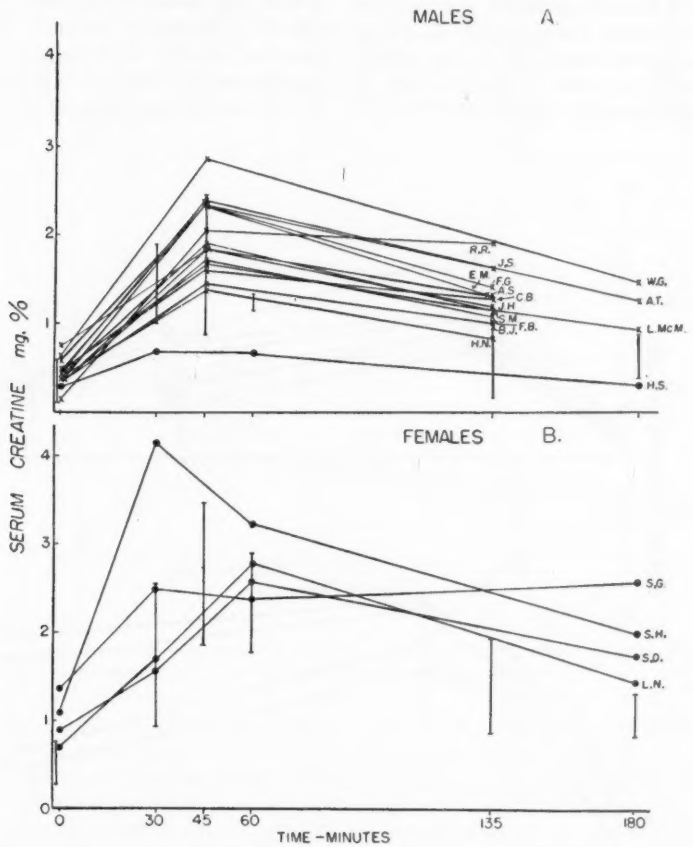

FIG. 2. UNTREATED HYPERTHYROIDISM

Vertical bars represent normal ranges from Figure 1. Crosses (×) authors' cases. Circles (●) from Tierney and Peters (6). A. Males, B. Females. Comparison of the male normal and hyperthyroid group at 135 minutes indicates a very real difference in that application of the "t" test reveals a "p" value of 0.0001. At 180 minutes the difference is similarly real, the fact that "p" is 0.01 being attributable to the small number of cases (in this instance the case of Tierney and Peters represented by the solid circle was omitted from the analysis, perhaps unjustifiably). The difference in the female groups at 180 minutes is also real. In neither males nor females can significance be attached to the fasting, 30, 45, or 60 minute values.

metabolism suggested by Figure 3A is not persistent in all cases, since the final creatine values for both F. B. and L. McM. are higher than in Figure 3A and somewhat exceed the normal range after 30 and 31 days of therapy, respectively. The one patient showing a definitely abnormal pattern after 13 and 31 days' medication (B. J.—Table II) had received Lugol's solution for a prolonged period prior to hospital admission, and treatment with propyl thiouracil was started 25 days later.

Following thyroidectomy, all of the patterns become normal, even those checked as early as seven days after operation (Figure 4B).

Other disorders

Eleven studies performed in four cases with nontoxic adenomas of the thyroid, one suspected of thyroiditis, one suspected of hyperfunction of the gland following a previous thyroidectomy, one suspected of hyperthyroidism and subsequently found to have a malignant sarcoma, and one outpatient with neurosis suggestive of thyroid disease, are reproduced in Figure 5. Clinical data are given in Table III. Unfortunately, the diagnostic specificity of the tolerance technique is impugned by the fact that the curve of the most complex

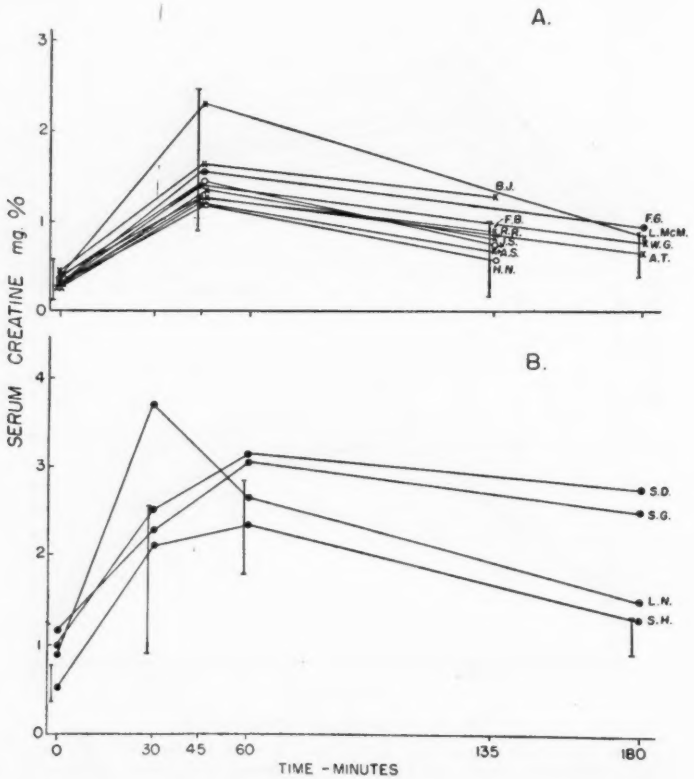

FIG. 3. EARLY THERAPY

Vertical lines represent normal range from Figure 1. Solid circles (●) indicate treatment with Lugol's solution, crosses (×) propyl thiouracil and open circles (○) therapy with both drugs. A. Males after ten days of medical treatment. B. Females after Lugol's solution, duration not stated, from Tierney and Peters (6).

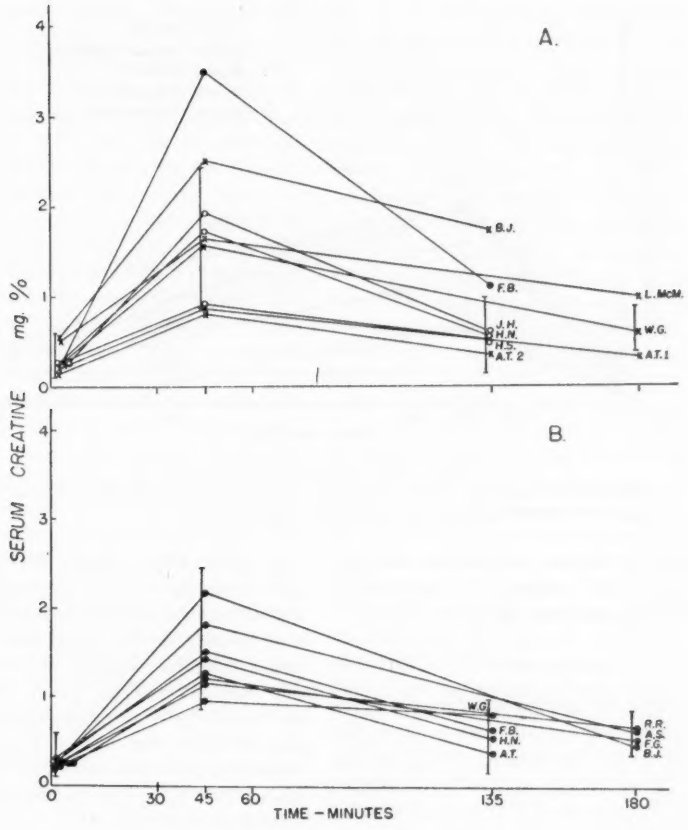

FIG. 4.

Vertical bars represent normal range from Figure 1. A. Later medical therapy and preoperative case (symbols as in Figure 3A). B. Postoperative cases.

clinical problem (H. W.—Table III) was equivocal while the serum iodine and basal metabolism were in agreement.. The abnormal pattern in the second study on H. C. can legitimately be assigned to physical deterioration associated with progression of his malignancy.

Serum creatinine values

Despite the dramatic alterations in serum creatine pattern during treatment of these cases, the creatinine remained relatively constant throughout. The range of values encountered, 0.77 – 1.62 mg. % (excepting values in the sarcoma patient in his terminal illness, H. C.—Table III) are in keeping with those previously reported in normals with this method (5, 6, 14). The maximum variation observed in any single tolerance was ± 0.36 mg. %, while in all but three cases the variation was ± 0.16 mg. % or less. Furthermore, there was probably no significant trend after treatment or operation, although four patients, A. S., W. G., R. R., and H. N., showed an increase of 0.22 mg. % or more in postoperative fasting creatinine over pretreatment levels, and only

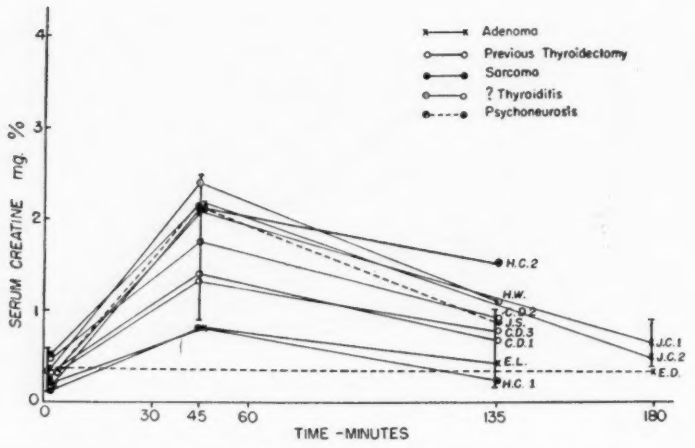

FIG. 5. NON-TOXIC THYROID DISEASES

Vertical lines represent normal range from Figure 1. Broken line (E. D.) indicates that the 45 minute specimen was technically unsatisfactory.

one patient, F. G., showed a significant decrease (0.13 mg. %). The constancy of creatinine in this regard has been noted by others (6, 15).

DISCUSSION

Examination of the overlap in values for males and females, normal or hyperthyroid, at the 30, 45, and 60 minute points in Figures 1 and 2 demonstrates that the alterations in creatine concentration at these intervals are of little or no diagnostic significance. Attention should, however, be drawn to the fact that in males serum concentrations, in this series (Figures 1–5) and in others (16), virtually never exceed 3.0 mg. %. In interpreting tolerance patterns the final (135 or 180 minute) specimen offers the most information and is the one which we have utilized in the interpretation of our findings. In fact, the data presented suggest that omission of the intermediate tolerance points is probably justified for practical purposes.

Females predominate in most reported case series of hyperthyroidism, and little information is available regarding creatine tolerance in males with this disease. The information obtained from the present study strongly suggests that the creatine disturbance in males is similar qualitatively but not quantitatively to that seen in women. In Graves' disease the male curves tend to become female, while female curves develop "super-feminine" characteristics, *i.e.*, an exaggeration of their normally-present inability to retain exogenous creatine in body depots. That this does not represent a complete blockage of creatine deposition and creatine-creatinine conversion in either sex has repeatedly been confirmed by measurements demonstrating that only a fraction of the administered creatine is wasted in the urine, usually less than 50%, even in the case of female hyperthyroid patients (2, 4, 6).

The fact that these shifts are approximately equal both directionally and quantitatively since the ranges of the 135 and 180 minute values for normal females correspond almost exactly to those for the hyperthyroid males (Figures 1B and 2A, respectively), suggests a cancellation of the anti-creatinuric effects of male androgenic secretion by the creatinuric action of excessive thyroid activity in this situation. Nevertheless, the mere fact that these antagonistic effects approximate one another in magnitude obviously fails to either confirm or deny existing concepts of thyroid-gonad interrelationships.

Creatine tolerance tests have been declared unsuited for use in the diagnosis of thyrotoxicosis (13), and so has measurement of endogenous creatinuria (17, 18). This is certainly true of en-

dogenous creatinuria in females. Normal women excrete creatine unpredictably but frequently, so that evaluation of its significance in individual instances is often impossible (6). In our relatively small number of cases the serum tolerance pattern in men has proved to be more specific, although the group is too small to exclude the possible existence of other types or stages of the disease in which normal curves might be found. Similarly, it is possible that the creatinuric "non-thyrotoxic hypertensives" reported by others (17) may display comparably reduced tolerances. To date, it has not proved possible for us to investigate the responses of such individuals.

Although in the few cases examined the creatine tolerance proved to be a valid index of thyroid function about as frequently as did the metabolic rate or the serum iodine, the number of patients is too small to justify such comparison at this time. However, it seems permissible to state that although determination of serum precipitable iodine and of basal metabolic rate are doubtless the most valuable procedures currently available for the diagnosis of abnormal function, the serum creatine tolerance is far more economical than the former, and proved to be a valid index in at least 19 of the 22 cases reported in this series. In instances where serum iodine data may be misleading (7–9) or where determination of the basal metabolic rate is technically impractical, the tolerance procedure may be of considerable clinical assistance in patients of either sex provided that the observer recognizes that such complicating conditions as cachexia, cardiac decompensation and acute infection may cause fundamental, and potentially confusing, disturbances of creatine metabolism. Furthermore, in certain respects the serum tolerance is preferable to the determination of postingestion creatinuria. Incomplete emptying of the bladder or the necessity of using catheters may be an obstacle to urinary studies. Preliminary investigations also suggest that serum tolerances may furnish more exact information in the differential diagnosis of other metabolic disorders (19).

As reflected in the creatine metabolism, response to therapy is prompt, and in most cases complete. The apparent failure of two patients to maintain the early improvement of creatine tolerance after moderately prolonged medical therapy (F. B. and L. McM.—Figure 4A), and the limited response of a third (B. J.), who had had a prolonged course of Lugol's solution prior to hospitalization, are singularly reminiscent of the once-popular concept of "iodine escape." It would however, obviously be improper to attach significance to changes which are quantitatively so small. Comparison with response to therapy in females (Figure 3B) is not justified since no data are available regarding the clinical or metabolic status of the female series. The evidence suggests that the female response is less complete or less prompt. On the other hand, it may have merely been less permanent, a possibility suggested by the data depicted in Figure 4A.

On comparing the effects of therapy depicted in Figure 3A with the clinical and laboratory findings in Table II, one finds that in most instances the basal metabolism and pulse rate were still elevated, although creatine tolerances were improved. It would be unwise, in view of our ignorance of the mechanisms involved, to attempt a physiologic interpretation of this fact. However, clinical experience furnishes a comparable phenomenon in that hyperthyroid patients frequently admit to subjective improvement during the early days of therapy before objective changes are detectable. Others have related the abnormalities in creatine metabolism in Graves' disease to the myasthenia seen in these patients (3, 4), and it seems logical to propose that the increased well-being reported by these individuals may be an expression of this early return of the ability to deposit creatine in the normal manner.

Patients with hypothyroid states (16) have failed to reveal as clear-cut a pattern as that observed in this study, and further data must be collected before definitive material can be reported. The tolerance patterns in hypermetabolic leukemia are inconstant, and often bizarre. Preliminary data suggest that the aberrations noted are not the result of impaired renal function or of uric acid chromogenicity (20). Further investigations are in progress.

SUMMARY AND CONCLUSIONS

1. Serum creatine tolerance curves (1 gm. p.o.) offer a more reliable index of thyroid function in males than in females.

2. There is in most instances a prompt improvement in tolerance to or toward normal patterns early in the course of medical therapy.

3. Creatine tolerances promptly reverted to the normal pattern in all instances following thyroidectomy.

4. The procedure confirmed clinical opinions and other laboratory findings in 12 of 13 cases of hyperthyroidism as well as in seven out of eight cases of suspected or non-toxic thyroid disease. In one instance, the diagnosis remains in doubt, but the tolerance was probably not in agreement.

5. Serum creatinine levels are essentially normal in male hyperthyroidism, tend to rise following thyroidectomy, and do not fluctuate significantly (< 0.4 mg. %) during the course of a 2¼ or three hour test.

ACKNOWLEDGMENTS

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Mr. Carrol Weil performed the statistical analyses.

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THE OCCURRENCE OF DIURESIS IN HUMANS IN STRESSFUL SITUATIONS AND ITS POSSIBLE RELATION TO THE DIURESIS OF EARLY STARVATION

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It is a common clinical observation that persons involved in stressful situations associated with feelings of anxiety and apprehension may have frequency of urination. Whether this phenomenon is the result of the ingestion of water, reflex irritability of the bladder, or a true polyuria of undetermined origin has not been entirely clear. That stimuli acting through the central nervous system may affect the rate at which the kidney excretes urine is well known. Rydin and Verney (1, 2) have shown that a water diuresis in dogs may be inhibited by the stimulation of afferent nerves or by loud noises. Kelsall (3), Wolf (4), and Odell and Aragon (5) have demonstrated a similar phenomenon in humans in reaction to pain. On the other hand "emotional diuresis" has also been observed experimentally (6). Marx (7), Heilig and Hoff (8), and Hoff and Wermer (9), have reported the induction of diuresis by hypnotic suggestion. Marx later reported (10) that he had succeeded in conditioning one of four dogs to a reflex diuresis after sham drinking. Bykow and Alexejew-Berkmann (11) also reported a conditional reflex diuresis in dogs, and Eagle (12) was able to condition animals to a reflex inhibition of diuresis after the ingestion of water. Anderson (13) observed a soldier with an ureteral fistula from which an increased urine flow could be produced by various "psychic" stimuli, and Rooke (14) stated that he has repeatedly observed an increased flow from ureteral catheters when patients were told to think about drinking beer or tea. Sheba observed that during an eight hour period soldiers, in expectation of going "over the line," excreted as much as 4 liters of urine with a specific gravity near 1.000 (15). A diuresis has been observed to occur in persons with diabetes mellitus under circumstances of environmental stress (16, 17), and it is well known that a transient diuresis accompanies the first stages of a total fast (18, 19). Both of these phenomena are temporally associated with changes in fat and carbohydrate metabolism. The present study concerns the experimental production of a water diuresis in humans during stressful situations. To provide an indication of other metabolic changes occurring at the same time, simultaneous measurements of blood glucose and blood ketone bodies were made throughout the experimental period.

METHODS

Subjects for this study were patients from the Medical Clinic of the New York Hospital with minor disorders such as functional diarrhea and headache, and volunteers from the hospital staff. Subjects were selected who were known to be reliable and intelligent persons, and who had been observed to develop anxiety under certain circumstances, especially in connection with some of their personal conflicts. Such persons made up both the stress and control groups. In none of the subjects was there any evidence of renal disease or other pathological processes known to interfere with kidney function. The patients were subjected to a prolonged study which included a medical history, physical examination, and indicated laboratory procedures, as well as an evaluation of their personality structures, life histories, cultural backgrounds, present life situations, and significant conscious and unconscious conflicts. The manner in which these studies were made has been described elsewhere (16).

Experiments were performed in the morning after an overnight fast. Except as noted in the protocols, subjects had ingested no fluids since the evening before. Muscular activity prior to the experimental period was limited to that involved in walking or riding to the laboratory. No smoking was allowed during the experiment. On arising the subject noted the time at which he emptied his bladder; when he arrived at the laboratory he again emptied his bladder, and the time was recorded. Observations were made during three periods of approximately one hour thereafter. During the first and third hours the subject amused himself reading books or magazines, or was engaged in neutral discussion with the interviewer. During the middle hour he was engaged in a

vigorous discussion of topics of significant personal conflict known to be associated with strong and conflicting conscious and unconscious attitudes and feelings.

The reaction of each subject to the procedure was evaluated by observation of his behavior, including both overt manifestations such as facial expression, content of remarks, tone of voice, tears, tachycardia, and sweating, as well as the more subtle clues such as slips of the tongue, figures of speech, and fleeting gestures, which gained significance for the observer from his past familiarity with the subject. The statements of the subject during the interview were recorded, and at later interviews the data were supplemented by his recollections and associations concerning all three of the experimental hours. This precaution was necessary since it could not be assumed that because he was apparently quietly and contentedly reading a magazine during the control hours that he was therefore "relaxed" and not reacting to his environment with significant attitudes and emotions.

The control procedures which were carried out were identical to those described above except that no stressful topics were discussed. Because laboratory procedures such as these may in themselves have threatening implications for some people, and because subjects may themselves introduce a stressful stimulus into an intended control procedure through their own ruminations, the subjects for the control procedures were studied as carefully before, during, and after the procedure as the subjects who were exposed to stressful interviews, and their experiences were as carefully evaluated.

Urine chlorides were measured by the method of Sendroy (20). Blood and urine ketones were determined by a macro-modification of the micro-method of Greenberg and Lester (21–23). Urine glucose was measured by the method of Benedict (24). Blood glucose determinations were made on filtrates prepared by the method of Herbert and Bourne (25), using one of Benedict's copper reagents (26–28); this method closely approximates "true blood glucose," excluding other reducing substances. Urine specific gravity was determined with a calibrated clinical hygrometer. Eosinophile counts were made after the manner described by Thorn and associates (29).

RESULTS

Control Studies

Because major changes in physical activity and in posture are known to affect the rate of excre-

TABLE I

Rate of water excretion, cc/min.

Control studies

	Subj.	Sex	Age	Position and activity	Fluid intake	Before arrival	Observation periods			Δ cc/min.	Δ %
							1st hr.	2nd hr.	3rd hr.		
1.	XH	F	25	Standing and walking about	None	—	0.2	0.2	0.2	0.00	0
2.	D	F	22			—	0.4	0.4	0.3	+0.05	+14
3.	XA	F	23			—	0.6	0.7	2.1*	−0.65	−48
4.	D	F	22			0.9	0.4	0.3	0.3	−0.05	−14
5.	XG	M	21			1.1	1.2	1.2	1.1	+0.05	+ 4
6.	A	F	20			—	0.6	0.5	0.4	0.00	0
7.	C	M	31	Sitting; clerical work	None	0.4	0.5	0.5	0.4	+0.05	+10
8.	B	F	30			0.3	0.4	0.3	0.4	−0.10	−25
9.	XF	F	35			0.3	0.9*	1.3*	2.3*	−0.30	−19
10.	E	M	35			—	0.5	0.9*	1.1*	+0.10	+12
11.	B	F	30			—	0.5	0.3	0.2	−0.05	−11
12.	AD	F	60	Lying in bed	None	1.0	0.7	0.7	0.7	0.00	0
13.	XC	F	28			0.6	0.6	0.5	0.7	−0.15	−23
14.	AX	F	42	Sitting, 1st and 3rd hrs.; lying, 2nd hr.	None	0.6	0.4	0.3	0.2	0.0	0
15.	AW	M	45		None	0.6	0.9	0.9	0.6	+0.15	+20
16.	XJ	F	29		None	0.5	0.8	0.7	0.5	+0.05	+ 8
17.	C	M	31		60 cc i.v. 2nd hr.	—	0.5	0.6	0.5	+0.10	+20
18.	H	F	27		60 cc i.v. 2nd hr.	0.8	0.8	1.0	0.9	+0.15	+18
19.	XD	F	40		60 cc i.v. 2nd hr.	1.8	1.1	0.8	0.7	−0.10	−11
20.	XE	F	30		60 cc i.v. 2nd hr.	—	0.5	0.7	0.7	+0.10	+17
21.	B	F	30		125 cc i.v. 2nd hr.	0.6	0.6	0.6	0.7	−0.05	− 8
22.	XM	M	30		125 cc i.v. 2nd hr.	0.4	0.4	0.4	0.5	−0.05	−11
23.	XN	F	40		125 cc i.v. 2nd hr.	0.6	0.7	0.8	1.3*	−0.20	−20
24.	E	M	35		125 cc i.v. 2nd hr.	1.0	0.6	0.9	0.8	+0.20	+22

Δ cc/min., mean: − 0.029 Standard error of mean ± 0.036
Δ %, mean: − 0.04 Standard error of mean ± 3.6

* Figures marked with an asterisk represent hours during which subjects experienced marked hunger.

"Delta" in each control study represents the difference between the values obtained during the first and third hours and those obtained during the interview hour. It is obtained by subtracting the average of the values obtained during the first and third hours from that obtained during the second hour.

TABLE II

Rate of water excretion, cc/min.

Stress studies
(stressful interview 2nd hr.)

	Subj.	Sex	Age	Position and activity	Fluid intake	Before arrival	Observation periods			Δ cc/min.	Δ %
							1st hr.	2nd hr.	3rd hr.		
1.	XI	F	32		None	0.3	**0.8**	**2.1**	0.6	+1.40	+175
2.	AX	F	42		None	0.2	**2.7**	**2.0**	0.5	+2.20	+440
3.	BF	M	15		None	0.3	0.4	0.8	1.0	+0.10	+ 14
4.	AW	M	45		None	**2.3**	**2.5**	**1.5**	0.8	+1.70	+212
5.	XJ	F	29		None	1.0	1.2	**2.7**	1.0	+1.60	+144
6.	XK	F	38	Sitting, 1st and	None	0.9	**3.3**	**4.5**	1.3	+3.20	+246
7.	XJ	F	29	3rd hrs.; ly-	None	**2.6**	**1.6**	**3.0**	0.6	+2.40	+400
8.	XO	F	40	ing, 2nd hr.	None	**1.4**	**2.5**	**1.8**	0.9	+1.60	+178
9.	XK	F	38		None	0.3	**1.2**	**3.2**	1.2	+2.00	+167
10.	H	F	27		40 cc i.v. 2nd hr.	—	0.8	**2.9**	1.5	+1.75	+152
11.	G	F	36		60 cc i.v. 2nd hr.	—	1.1	**2.1**	0.2	+1.45	+223
12.	F	F	33		200 cc P.O. 1st–3rd hrs. 100 cc i.v. 2nd hr.	—	1.0	**5.1**	2.0	+3.60	+240

Δ cc/min., mean: + 1.92 Standard error of mean ± 0.26
Δ %, mean: + 216 Standard error of mean ± 32.9

Δ cc/min., mean difference between means of stress and control studies,
1.95 ± 0.26 $P < 0.001$

Δ %, mean difference between means of stress and control studies,
216 ± 32.9 $P < 0.01$

Values in bold type are those obtained during hours in which the subjects were anxious and apprehensive.
In stress studies "Delta" was obtained by subtracting the average of the values obtained during the hours in which the subjects were not apprehensive from the highest value during the hours in which they were anxious and apprehensive.

tion of water, and because it is impossible to prevent some change in position and activity from occurring in patients during interviews of this sort, control studies were made on subjects in a wide variety of positions and during various degrees of light activity. As seen in Table I, subjects were tested while lying in bed, sitting, standing, and walking about, doing laboratory or clerical work, all or part of the time. It was found that such minor variations in activity had no noticeable effect upon the urine output of these subjects. In those in whom an accurate estimation of the rate of urine formation could be made from the time they arose until they arrived at the laboratory, it was found that the rate of urine formation prior to the test was not significantly different from that during the three hours of observation. Because of the known diurnal variations in human urine volume, all of the studies were made between 9 a.m. and 1 p.m., beginning two to three hours after the subject had arisen from bed. The only exceptions to this were the two studies (stress and control) on subject H and the stress study on subject F, which were made in the afternoon.

In a few of the subjects, in order to facilitate the collection of blood samples at frequent intervals during the stress interview, a continuous slow intravenous drip of 0.9% saline at the rate of approximately 1 cc/min. was maintained during this time through an indwelling needle. The total amount administered was 60 to 100 cc. In view of the possible diuretic effect of this procedure similar intravenous injections were given to some of the control subjects. In these individuals, however, diuresis was never observed with as much as 125 cc of saline administered intravenously during the course of an hour while the subjects were supine.

In none of the control subjects was there significant change in the urine specific gravity or in the rate of excretion of water, chlorides, and ketone bodies, during the hour of the neutral interview as compared to the control hours, although a slight and steady rise or fall in the various excretion rates during the three hours sometimes occurred. The rate of water excretion lay between 0.2 and 1.1 cc/min. (Table I), the rate of chloride excretion lay between 2.1 and 9.7 mg/min., and the rate of ketone excretion lay between 0.005 and 0.049 mg/min. (expressed as acetone recovered). A 10%

to 40% fall in circulating eosinophiles occurred in most subjects during the course of the morning.

In 20 of the 24 studies these excretion rates, as well as the urine specific gravity, changed very little throughout the morning (Table III). The concentration of the ketone bodies and of glucose in the blood of these subjects also remained approximately constant. This point is not illustrated in the tables, but is dealt with elsewhere (23, 30). The protocol of one of these studies is diagrammed in Figure 1. The slight but steady fall in the blood glucose and the slight but steady rise in the circulating ketones which usually occur during a morning's fast were accompanied by relatively little change in the rate of excretion of water, chlorides, and ketones, and no consistent change in the concentration of these substances in the urine. In four of the subjects, however, there occurred an increase in the urine volume during the later

TABLE III

Summary of changes observed in other urinary constituents

Chlorides (expressed as NaCl)

Concentrations:

Δ mg %, mean of control studies: + 50 ± 33
Δ mg %, mean of stress studies: −377 ± 75
Mean difference: 427 ± 82 P < 0.005

Rates:

Δ mg/min., mean of control studies: 0.0 ± 0.5
Δ mg/min., mean of stress studies: + 0.8 ± 0.5
Mean difference: 0.8 ± 0.7 P = 0.3

Ketones (expressed as acetone recovered)

Concentrations:

Δ mg %, mean of control studies: + 0.36 ± 0.31
Δ mg %, mean of stress studies: − 0.21 ± 0.29
Mean difference: 0.57 ± 0.42 P = 0.2

Rates:

Δ mg/min., mean of control studies: + 0.0031 ± 0.0030
Δ mg/min., mean of stress studies: + 0.0191 ± 0.0082
Mean difference: 0.0160 ± 0.0088 P = 0.1

Specific Gravity:

Δ Sp.Gr., mean of control studies: + 0.0002 ± 0.0004
Δ Sp.Gr., mean of stress studies: − 0.0078 ± 0.0015
Mean difference: 0.0080 ± 0.0015 P < 0.002

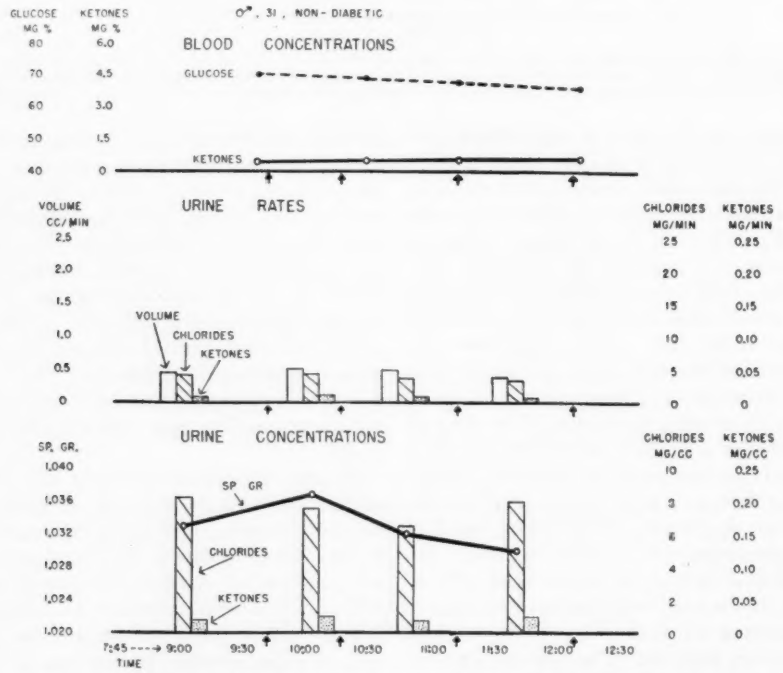

FIG. 1. DIAGRAM OF A CONTROL STUDY, SHOWING RELATIVELY LITTLE CHANGE IN CONCENTRATION OF BLOOD GLUCOSE AND KETONES, IN THE RATE OF EXCRETION OF WATER, CHLORIDES, AND KETONES, AND IN THE CONCENTRATION OF THESE SUBSTANCES IN THE URINE

Arrows on the diagram represent collection points. Interview took place during second hour.

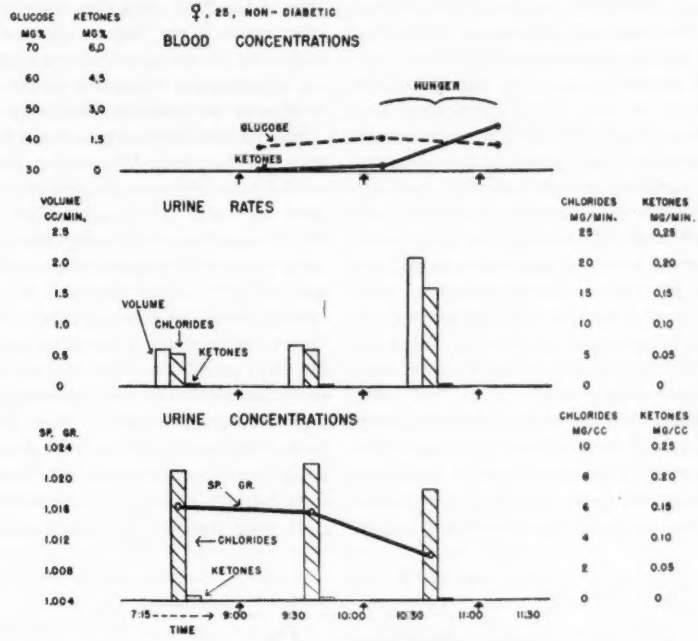

FIG. 2. DIAGRAM OF A CONTROL STUDY ON A SUBJECT WHO BECAME HUNGRY DURING THE LATER PART OF THE MORNING, SHOWING THE RISE IN BLOOD KETONES AND THE DIURESIS WHICH OCCURRED AT THE SAME TIME

Subject was standing, walking about, doing routine laboratory work throughout the morning.

stages of the morning. This could not be related to changes in activity, position, or fluid intake, but it was associated with a pronounced hunger and a feeling of generalized tension. The rise in urine volume was accompanied by a fall in the specific gravity of the urine and in the concentration of ketones; because of this fall in concentration, the rate of ketone excretion was relatively unchanged. The chloride concentration fell relatively less, if at all; because of this the diuresis was accompanied by a rise in chloride excretion. This diuresis was regularly accompanied by a rise in the concentration of the blood ketones, a fall in the blood glucose concentration, and a fall in the number of circulating eosinophiles. The changes in the blood constituents did not appear to be the cause of the diuresis, however, since they occurred also in some subjects who had no diuresis. The protocol of one of these studies is diagrammed in Figure 2.

Stress Studies

In 11 out of 12 subjects in whom intense conflicts were aroused a diuresis occurred (Table II). This was a diuresis of water, accompanied by a fall in the specific gravity of the urine and in the concentration of chloride (Table III). Because of the fall in chloride concentration, the rate of chloride excretion did not consistently change, although in some cases it rose. Accompanying the diuresis there was regularly a fall in the blood glucose and a rise in the blood ketones. A 50% to 80% fall in the number of circulating eosinophiles occurred in most of the stress experiments. In some instances the concentration of ketones in the urine fell, and the rate of ketone excretion remained relatively constant; in others, in whom there was a marked rise in the blood ketones, the ketone concentration in the urine and the rate of ketone

excretion rose. The blood glucose and ketone and eosinophile levels are not included in the tables, but are dealt with in other papers (*23*, 30).

Periods of increased urine flow coincided with periods of more or less overt apprehension and anxiety in the subjects, and appeared to be related to specific personal conflicts only in the degree to which such conflicts aroused anxiety. Subject BF, for example, was notably depressed and angered during the discussion of the cruel treatment he received from his oppressive father, but we could find no evidence that the interview made him anxious. He was the only subject in the stress group who developed no diuresis. All of the other subjects were anxious during the interview, and had elevated rates of urine output during this time. But the discussion in itself was obviously not the only cause of the diuresis. It appeared that any anxiety-arousing situation might be associated with a diuresis. Subjects who became apprehensive when they were told that they would have a "special test," or when they were waiting for the interview to begin, had an elevated urine output during the initial hour; whereas those who were not apprehensive before the interview period had no diuresis until the interview began.

Subject AW, for example, was a business executive who had been hospitalized because of precordial pain, and who thought that he had a coronary occlusion. Although he had been told that this was not the case, he suspected that the physicians were only trying to reassure him. When he was told that on the next day he would have a "special test" he was suspicious that it was a "heart test" and would reveal severe heart disease. He slept poorly the night before, was apprehensive when awakened, and remained so all morning until the interview ended. At a later date, when he realized that he had no heart disease, and when the procedure represented no threat to him, he went through a physically identical experiment in a relatively tranquil state, and had no diuresis.

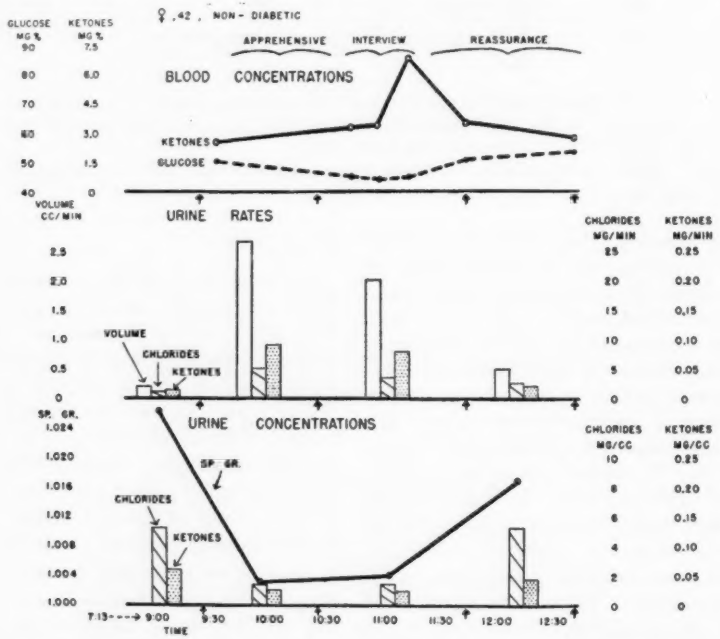

FIG. 3. THE DIURESIS ASSOCIATED WITH A STRESSFUL LIFE SITUATION, SHOWING ACCOMPANYING CHANGES IN BLOOD GLUCOSE AND KETONE CONCENTRATION, AND IN THE CONCENTRATION OF CHLORIDES, KETONES, AND WATER IN THE URINE

In describing the situations in which their urine flow was elevated, the subjects used terms such as "I didn't know what was going to happen," "I was worried about what you were going to do to me," "The way you acted made me nervous," or "I was nervous talking about mother like that." They were moderately to markedly apprehensive, rather than overwhelmingly afraid. None of them hyperventilated.

Illustrative of these findings is the case of a 42 year old business woman, a divorcee who complained of weakness and fatigue (Figure 3). On the day of the procedure she came in slightly late, expressing apprehension about what would be done. She quickly settled herself comfortably, however, and talked in a friendly and ostensibly calm manner about minor experiences of the day.

At the beginning of the second hour when she was asked to lie on the examining table her anxiety and apprehension recurred and became evidently more intense as the indwelling obturator needle was placed in her vein. "What are you doing?" she said with obvious alarm. Within approximately 15 minutes, in association with a discussion of frustrations in her job and resentment of her mother whom she was required to support, she expressed hostility mingled with depressive preoccupations. Finally, the evidence of depression predominated and she said, "You are going to an awful lot of trouble for me and I don't deserve it, I'm not used to that kind of treatment and I feel guilty. I'm not worth it." Throughout this middle hour the underlying anxiety and apprehension persisted, and although her manner was often deprecatory, she watched the periodic blood drawing procedure uncomfortably and said, "When do I get a transfusion?"

During the last hour she sat quietly, at first musing over depressive thoughts, and then became increasingly impatient to get done with the procedure and back to work. The findings in this case are presented diagrammatically in Figure 3, which shows a steep rise in urine flow during the period marked predominantly by anxiety and apprehension, associated with a precipitous fall in specific gravity and chloride concentration. It is also of interest that a rise in the concentration of blood ketones corresponded with the period of maximal evidence of resentment. That the changes in blood glucose and ketone concentration were not causally related to the changes in urine output are apparent from the fact that the diuresis took place before any significant change in the blood constituents occurred.

DISCUSSION

It is apparent that the rate of excretion of water in humans is sufficiently under the control of the central nervous system to allow the initiation of a diuresis as great as 4.5 cc/min. in individuals who have received no fluids during the preceding 12 hours.

It has long been known that a transient diuresis may occur in some subjects during the earliest stages of a total fast (18, 31). This diuresis quickly subsides if the subject is totally starved, but in the partially starved person polyuria may be an outstanding symptom (19). It has been suggested that this diuresis may be advantageous in reducing the renal osmotic work and thereby reducing the consumption of energy producing substances by the kidney (32). Winkler and associates (31) felt that it might be the result of increased nitrogen excretion. However, its appearance in conjunction with a rise in the blood ketones and a fall in the blood glucose suggests that it may be a result of the metabolic rearrangements which take place as the organism shifts from carbohydrate to fat as a primary source of energy, as Gamble and his coworkers (18) first suggested. Presumably such a shift may make available a slight excess of body water which demands excretion. If these conjectures are correct, it may be that the diuresis which these subjects developed in a stressful situation is a part of a generalized metabolic response which is set in motion by an implied threat to the integrity of the organism.

The mechanism by which these changes are brought about is not clear. It seems possible that they may be the result of an inhibition of the secretion of the antidiuretic hormone by the posterior pituitary, leading to a decrease in the reabsorption of water by the distal tubule. The fall in circulating eosinophiles which coincided with the diuresis suggests that there may also have been an increased elaboration of the adrenal cortico-steroids. Circulating epinephrine would seem to be an inadequate explanation, because of the inconstant effect of this hormone on urine output, and the absence of obvious epinephrine effects in some

subjects who had diuresis. Furthermore it cannot be ruled out that these changes in water excretion were the result of changes in renal hemodynamics. It has been shown that stressful situations may result in changes in renal blood flow, in glomerular filtration, and in the filtration fraction (4, 33). Although it is generally felt that changes in renal blood flow are not reflected in changes in urinary volume except under extreme circumstances (34), such conclusions have been drawn largely from clearance studies which were made at rates of urine flow (3–10 cc/min.) not usually seen in humans except after a great deal of water has been ingested. Other studies, made at lower rates of urine formation, suggest that under such conditions the volume of urine flow may be related to the renal blood flow and glomerular filtration (35).

In any case it is not possible to attribute the polyuria of stressful life situations entirely to the increased ingestion of water. It may even be that the increased water ingestion which occurs under such circumstances is the result of the thirst engendered by the diuresis. This phenomenon appears to be of special significance in individuals suffering from diabetes mellitus. In them it has been found that a profound diuresis occurring in reaction to life stress may be of great importance in the pathogenesis of the dehydration which is associated with some episodes of diabetic ketoacidosis. This subject will be discussed in a subsequent paper.

CONCLUSIONS

Stimuli, in the form of threatening life situations, acting through the highest integrative levels of the central nervous system, may lead to a spontaneous diuresis in hydropenic humans. This diuresis is characterized by a 200% to 500% increase in the urine volume, accompanied by a fall in the specific gravity of the urine, and a fall in the concentration chlorides. It is commonly accompanied by a rise in the concentration of ketone bodies in the blood and a moderate fall in the level of the blood glucose, but does not appear to be caused by changes in these blood constituents. In the presence of a large increase in the concentration of ketone bodies, the rate of ketone excretion in the urine may also rise; otherwise, there may be no increase in ketone excretion in association with the diuresis. Feelings of anxiety and apprehension were found to occur in company with the diuresis described.

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STUDIES IN DIABETES MELLITUS. II. THE OCCURRENCE OF A DIURESIS IN DIABETIC PERSONS EXPOSED TO STRESSFUL LIFE SITUATIONS, WITH EXPERIMENTAL OBSERVATIONS ON ITS RELATION TO THE CONCENTRATION OF GLUCOSE IN BLOOD AND URINE [1]

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It has been known for many years that the administration of large amounts of glucose to persons with diabetes mellitus may lead to a diuresis. Recent work has amply demonstrated that this effect is attributable to the fact that in the presence of high concentrations of urinary glucose or any other solute there is a limit to the amount of water the renal tubule can absorb—a point where the osmotic pressure of the urine outweighs the work capacity of the tubular epithelium. Brodsky, Rapoport, and West (1) have shown that in this respect the kidney of the diabetic behaves toward glucose in the same manner as the kidney of the non-diabetic individual.

However, there is an increasing body of evidence which suggests that not all of the polyuria which occurs in diabetic persons can be attributed to an increase in the amount of glucose excreted. For example, Tolstoi and Weber (2) observed that diabetic individuals on a metabolism ward, with a fixed regimen of activity, diet, fluid intake, and insulin, excreted widely variable amounts of glucose and water from day to day. The reasons for the fluctuations were not apparent. Meyer, Bollmeier, and Alexander (3), observing two diabetic patients during the course of psychoanalytic treatment, found that these persons developed an increased excretion of glucose and water during acute conflict situations, and that this diuresis could not be related to changes in food intake. More recently, during the course of prolonged observations upon persons with diabetes mellitus of all grades of severity, the present authors (4–7) have repeatedly observed an increase in water and glucose excretion, sometimes associated with ketosis and coma, occurring in association with stressful life situations, and not attributable to infection, trauma, or changes in diet, insulin, or activity. During short term experimental observations (8) it was found that such stresses may induce a marked increase in the ketonemia of both diabetic and non-diabetic persons and significant changes in their blood glucose levels, and that these metabolic changes are frequently accompanied by diuresis. Experimental investigation of this diuresis in non-diabetic persons (9) showed it to be characterized by an increase of up to 500% in the rate of water excretion, accompanied by a fall in the specific gravity of the urine and in the concentration of chlorides and ketone bodies. The changes in the constituents of both the blood and the urine were found to be quite similar to those which take place during the diuresis which occurs during the early stages of a total fast.

The present report describes the results of a study of the phenomenon of "stress diuresis" in persons with diabetes mellitus.

METHODS

Subjects for this study were patients from the Diabetic Clinic of the New York Hospital. The group contained proportionately more persons with severe and labile diabetes than is found in the diabetic population as a whole, but in other respects it was unselected. None of the subjects showed evidence of functional renal impairment, but the possibility that some of them had structural lesions of the kidney cannot be ruled out, inasmuch as several had had diabetes mellitus for a decade or more and showed evidence of generalized arteriosclerosis, diabetic reti-

[1] The authors are indebted to Dr. Edward Tolstoi for making available the facilities of the Diabetic Clinic of the New York Hospital for this study, and for his helpful criticism of this work.

nopathy, or transient episodes of albuminuria. However, a fair proportion of young people with diabetes of recent onset and no evidence of vascular disease were included in the group, and it is probably safe to assume that many of these had no lesions in their kidneys. The patients were subjected to a prolonged study which included a medical history, physical examination, and appropriate laboratory procedures, as well as an evaluation of their personality structures, life histories, cultural backgrounds, present life situations, and significant conscious and unconscious conflicts. The manner in which these studies were made has been described elsewhere (4).

Experiments were performed in the morning after an overnight fast. Those who had been receiving insulin had been on syringe mixtures of Regular and Protamine Insulin usually in a 2:1 ratio, and had received no insulin for 24 hours. All had been consuming at least 125 gm of carbohydrate daily, and there had been no recent major dietary changes. One group of subjects (Table I) received nothing by mouth from the time they retired the night before, and nothing during the experimental period. A second group (Table II) received one glass of water (200 cc) on arising, two hours before the study began, and 200–250 cc per hour by mouth or vein during each experimental hour, as noted in the text. Muscular activity prior to the experimental period was limited to that involved in walking or riding to the laboratory. No smoking was allowed during the experiment. On arising the subject noted the time at which he emptied his bladder; when he arrived at the laboratory he again emptied his bladder, and the time was recorded. Observations were made during three periods of approximately one hour thereafter. Except where otherwise indicated, the procedure was as follows: During the first and third hours the subject "amused himself" reading books or magazines, or was engaged in neutral discussion with the interviewer. During the middle hour he was engaged in a vigorous discussion of topics of significant personal conflict known to be associated with strong and conflicting conscious and unconscious attitudes and feelings. The purpose of this interview was to create a situation which would arouse the feeling states and physiological responses which were usually associated with the important conflict situations in the patient's life.

The reaction of each subject to the procedure was evaluated by observation of his behavior, including both overt manifestations such as facial expression, content of remarks, tone of voice, tears, tachycardia, and sweating, as well as the more subtle clues such as slips of the tongue, figures of speech, and fleeting gestures, which gained significance for the observer from his past familiarity with the subject. The statements of the subject during the interview were recorded, and at later interviews the data were supplemented by his recollections and associations concerning all three of the experimental hours. This precaution was necessary since it could not be assumed that because he was apparently quietly and contentedly reading a magazine during the control hours he was therefore "relaxed" and not reacting to his environment with significant attitudes and emotions.

The control procedures which were carried out were identical with those described above except that no stressful topics were discussed. Because laboratory procedures such as these may in themselves have threatening implications for some people, and because subjects may themselves introduce a stressful stimulus into an intended control procedure through their own ruminations, the subjects for the control procedures were studied as carefully before, during, and after the procedure as the subjects who were exposed to stressful interviews, and their experiences were as carefully evaluated.

The chemical methods used have been outlined in previous papers (8, 9).

RESULTS

Control Studies

As in the studies on the non-diabetic subjects (9), it was found that the variations in posture and the minor variations in activity associated with the interview procedure had no significant effect upon the urine output. In order to facilitate the collection of blood samples at frequent intervals during the interview hour, some of the subjects received a continuous, slow intravenous drip of 0.9% saline during this period at the rate of approximately 1 cc/min. The total amount administered was 60–100 cc. This procedure, which had had no diuretic effect in non-diabetic persons, was likewise found to have no measurable diuretic effect in diabetics. There was no significant difference in the rate of excretion of urine during the hour of neutral interview (second hour) as compared to the average rate of excretion during the first and third hours (Table I). It was also found that there was no significant change in the urinary specific gravity, or in the rate of glucose (Table III), chloride (Table IV), or ketone excretion (Table V), during the interview hour. The rate of urine formation prior to the test was not significantly different from that during the three hours of observation.

The protocol of a control study is presented diagrammatically in Figure 1. At the outset of the study this subject's blood glucose concentration was 134 mg %, and he had 17 mg/cc of glucose in his urine. Subjects for the control studies showed various degrees of glycosuria from 0.0 to 100 mg/cc at the time of their arrival at the laboratory (Table III). Nevertheless, as may be seen here, the rate of water and chloride excretion of 11 out of 14 of them was within the same range as that

found in non-diabetics under similar circumstances (9); likewise their rates of excretion did not vary significantly throughout the morning. Even persons with a high concentration of glucose in their urine and a significant hyperglycemia were observed to develop no polyuria during a morning in which they were not under stress. Figure 2 is the diagram of a study on such a person (Subject

TABLE I

Rate of water excretion (cc/min.)

	Subject	Sex	Age	Insulin requirement	Before arrival	Observation periods			Δ cc./min.	Δ %
						1st	2nd	3rd		
				Control Studies						
1.	J*	F	42	10u		0.2	0.4	0.4	+0.1	+ 33
2.	K*	M	29	110u	0.5	0.6	0.5	0.5	−0.05	− 9
3.	I*	M	15	50u		0.5	0.7	0.7	+0.1	+ 17
4.	S*	F	50	Diet only	0.3	0.5	0.6	0.5	+0.1	+ 20
5.	AB*	M	50	28u	1.4	0.8	0.5	1.2	−0.5	− 50
6.	I	M	15	50u	0.5	0.9	0.7	0.6	−0.05	− 7
7.	U*	F	20	100u	0.2	0.3	0.4	0.4	+0.05	+ 14
8.	P	M	16	60u	0.3	0.5	0.5	0.5	0.0	0
9.	Y	F	16	90u	0.9	1.5‡	1.7‡	1.8‡	+0.05	+ 3
10.	BL	F	38	10u	0.8	0.9	0.8	0.9	−0.1	− 11
11.	Z†	M	20	100u	4.2	4.6	3.3	2.6	−0.3	− 8
12.	BC	F	17	75u	0.2	0.3	0.3	0.3	0.0	0
13.	AJ	F	34	30u	0.7	2.0‡	2.4‡ 2.5‡	2.8‡	0.0	0
14.	O	F	18	100u	0.6	0.5	0.5	0.3	+0.1	+ 25
	Δ cc/min., mean = − 0.04						Standard error of mean ± 0.05			
	Δ % change, mean = − 2						Standard error of mean ± 5			
				Stress Studies§						
1.	AC*	F	52	45u	1.0	**2.0**	**1.8**	0.5	+1.5	+300
2.	Y*	F	16	90u	1.9	**4.5**	**4.7**	3.3	+1.4	+ 42
3.	N*	F	55	10u		0.7	**0.5**k	0.4	−0.05	− 9
4.	K*	M	29	110u	1.0	**1.7**	**4.4**	1.2	+3.2	+267
5.	J	F	42	10u	0.4	**1.1**	**0.9**	0.7	+0.4	+ 57
6.	AC*	F	52	45u	0.3	**0.7**	**1.7 3.2**	0.5	+2.7	+540
7.	BC	F	17	75u	0.4	0.5	**0.5**k	0.4	+0.05	+ 11
8.	AN	F	40	Diet only	1.0	1.1	**2.0 1.9**	0.6	+1.2	+141
9.	BH	M	29	30u	0.7	0.8	**1.2**	**1.5**	+0.7	+ 88
10.	Y	F	16	90u	0.7	**2.9**	**2.8**	1.6	+1.3	+ 81
11.	BI	F	44	Diet only	1.2	1.0	**0.9**k	0.7	+0.05	+ 6
12.	AM	F	20	110u		**1.3**	**1.7**	0.5	+1.2	+340
13.	Y	F	16	90u	1.1	**2.8**	**2.7**	1.1	+1.7	+154
14.	AB*	M	50	28u	0.6	**2.1**	**0.5**k	1.1	+1.0	+ 91
	Δ cc/min., mean = + 1.17						Standard error of mean ± 0.27			
	Δ % change, mean = + 149						Standard error of mean ± 42			
	Δ cc/min., mean difference between stress and control studies 1.21 ± 0.27 P < 0.01									
	Δ % change, mean difference between stress and control studies 149 ± 42 P < 0.01									

* Subjects received 60–100 cc 0.9% saline i.v. during 2nd observation period.
† This subject was in moderately severe ketosis on arrival (blood ketones 23.8 mg %).
‡ Subjects experienced hunger during these hours.
§ Values in bold type were obtained during stress periods.
Stress periods marked "k" were accompanied by rise in blood ketones despite absence of diuresis. See text.

Derivation of delta:

Δ represents the difference between values obtained during the control periods and those obtained during the interview or stress periods. In control studies, Δ is obtained by subtracting the average of the values obtained during the two control hours from that obtained during the hour of neutral interview (2nd hour). In stress studies, Δ is obtained by subtracting the average of the values obtained during the control hours from the highest value obtained during a stress period. Values obtained "on arrival" were not included as control values except in the few instances where no other control values were available. In a few studies the interview period (2nd hour) was prolonged to include two collection periods of approximately 45 minutes each. In such cases both of the values obtained are printed in the table under "2nd observation period."

TABLE II

Rate of water excretion (cc/min.)

Subject	Sex	Age	Insulin requirement	Before arrival	Observation periods			Δ cc/min.	Δ %
					1st	2nd	3rd		
Control Studies—Fluid intake 250 cc/hr.									
V	F	35	40u		1.3	2.4*	3.3*	+0.1	+ 4
T	M	55	Diet only		0.4	0.6	0.5	+0.1	+ 33
P	M	16	60u		1.1	1.2	1.1	+0.1	+ 8
U	F	20	100u		1.0	2.4*	2.9*	+0.5	+ 30
R	F	21	40u		2.0	2.3	2.2	+0.2	+ 10
Δ cc/min., mean = + 0.20					Standard error of mean ± 0.08				
Δ % change, mean = + 17					Standard error of mean ± 6				
Stress Studies†—Fluid intake up to 250 cc/hr.									
U	F	20	100u		**3.8**	**4.7**	2.1	+2.6	+124
Q	F	14	60u		**0.9**	**0.4**	"none"		
V	F	35	40u		1.1	**5.2**	**2.3**	+4.1	+373
R	F	21	40u		**2.1**	**2.1**[k]	2.6*	−0.5	− 19
BE	F	47	Diet only	1.0	**4.2**	**6.1**	**5.1**	+5.1	+510
N	F	55	10u	0.4	**0.4**	**0.5**[k]	0.4	+0.1	+ 25
P	M	16	60u		0.7	**1.1**	0.5	+0.5	+ 83
I	M	15	50u		0.6	**2.6 1.8**	0.5	+2.1	+373
X	F	55	Diet only		0.5	**1.6**	0.9	+0.7	+129
Δ cc/min., mean = + 1.63					Standard error of mean ± 0.66				
Δ % change, mean = + 177					Standard error of mean ± 64				

Δ cc/min., mean difference between stress and control studies 1.43 ± 0.66 $0.1 > P > 0.05$

Δ % change, mean difference between stress and control studies 160 ± 64 $0.05 > P > 0.02$

* Subjects experienced hunger during these hours.

† Values in bold type obtained during stress. See Table I footnote for "k".

I) who had a blood glucose of 286 mg %, and 73.4 mg/cc of glucose in his urine, yet excreted water, chlorides, and ketones at a normal rate throughout a three hour period.

Factors which modified results of control studies

Two of the control subjects developed a gradual increase in the rate of excretion of all of the measured urinary constituents during the morning, accompanied by a slight rise in the blood ketone level and a fall in the blood glucose as exemplified in Figure 3. It is of interest that both of these persons developed fairly marked hunger during the morning, accompanied by increasing tension. In some non-diabetic persons under the same circumstances a diuresis associated with similar changes in the blood constituents was observed (9). This phenomenon in diabetic persons resembles the "starvation diuresis" which accompanies the early stages of a total fast in a non-diabetic person. The sensation of hunger appeared to be neither the determining factor nor a necessary accompaniment of these changes; regardless of hunger certain individuals apparently react to the withholding of food with metabolic alterations which include a diuresis.

One of the control studies was done upon a patient (Subject Z) who was in a moderately severe state of ketosis when he arrived at the laboratory. He had had a verbal conflict with his father the evening before—an occurrence which in the past had been followed repeatedly by episodes of ketosis (4). His initial blood ketone concentration was 23.8 mg %, his blood glucose was 206 mg %, and he was excreting water at the rate of 4.2 cc/min. and glucose at the rate of 319 mg/min. He was tense, resentful, and somewhat depressed. No attempt was made to reassure him or to allow him to express his feelings; instead he was lightly diverted in friendly surroundings during the morning. Under these circumstances his polyuria gradually diminished in intensity, but there was no significant change in his blood ketone level.

Another subject (AB) who had a high concen-

TABLE III

Glucose excretion

	Subject	Rate of excretion						Concentration				
		Before arrival	Observation periods			Δ mg/min.	Δ %	Before arrival	Observation periods			Δ mg/cc
			1st	2nd	3rd				1st	2nd	3rd	
						Control Studies						
1.	J	0	0	0	0	0	0	0	0	0	0	0
2.	K	0	0	0	0	0	0	0	0	0	0	0
3.	I	0	0	0	0	0	0	0	0	0	0	0
4.	S	1	4	8	7	+ 3	+ 60	3	8	11	12	+ 1
5.	AB	88	50	30	72	− 31	− 58	64	63	57	61	− 5
6.	I	36	65	56	34	+ 11	+ 24	73	70	75	53	+14
7.	U	0	0	0	0	0	0	0	0	0	0	0
8.	P	6	9	6	5	− 1	− 14	17	17	13	10	− 5
9.	Y	55	105*	118*	113*	+ 9	+ 8	59	70*	69*	64*	+ 2
10.	BL	0	0	0	0	0	0	0	0	0	0	0
11.	Z†	319	339	237	212	− 38	− 14	76	74	72	69	+ .5
12.	BC	2	3	2	2	− 0.5	− 25	9	10	7	7	− 1.1
13.	AJ	55	131*	152* 159*	179*	+ 1	+ 1	77	64*	63* 63*	62*	0.0
14.	O	0	0	0	0	0	0	0	0	0	0	0
15.	V		100	170*	240*	0	0		77	72*	74*	+ 2
16.	T		5	7	6	+ 1	+ 17		12	12	11	+ .5
17.	P		46	23	15	− 7	− 23	49	41	19	13	+ 8
18.	U		52	64*	76*	0	0	62	50	25*	25*	−12
19.	R	96	165	207	188	+ 31	+ 18	100	83	93	86	+ 8

Mean values for subjects with glycosuria:

Δ mg/min., mean = − 1.7 — Standard error of mean ± 4.8
Δ % change, mean = − 0.5 — Standard error of mean ± 8
Δ mg/cc, mean = + 1.3 — Standard error of mean ± 1.8

Subjects Having Stress Diuresis‡

1.	AC	15	**10**	**10**	5	+ 5	+100	14	**5**	**6**	8	− 2
2.	Y	189	**490**	**485**	326	+164	+ 50	95	**108**	**97**	98	+10
3.	K	11	**31**	**99**	28	+ 71	+254	11	**18**	**23**	24	− 1
4.	J	18	**40**	**22**	10	+ 30	+300	44	**39**	**26**	14	+25
5.	AC	3	6	**0 0**	0	− 3		12	7	**0 0**	0	− 3
6.	AN	0	0	**0 0**	0	0		0	0	**0 0**	0	0
7.	BH	0	5	**10**	**20**	+ 15	+300	0	3	**9**	**14**	+11
8.	Y	50	**225**	**250**	140	+110	+ 79	78	**78**	**89**	85	+ 4
9.	AM	0	0	0	0	0		0	0	0	0	0
10.	Y	62	**210**	**190**	87	+123	+142	59	**77**	**72**	77	0
11.	AB	6	**17**	4	7	+ 10	+143	9	8	9	7	+ 1
12.	U		**255**	**376**	134	+242	+180		66	**80**	72	+ 8
13.	V	0	**0**	**0**	0	0	0	0	**0**	**0**	0	0
14.	BE	0	**0**	**0**	**0**	0	0	0	**0**	**0**	0	0
15.	P		46	**80**	31	+ 42	+ 55	62	70	**72**	67	+ 3
16.	I		17	**80 50**	16	+ 63	+394	40	29	**31 27**	27	+ 3
17.	X	0	0	**0**	0	0	0	0	0	**0**	0	0

Mean values for subjects with glycosuria:

Δ mg/min., mean = + 80 — Standard error of mean ± 22.5
Δ % change, mean = + 182 — Standard error of mean ± 35
Δ mg/cc, mean = 5.6 — Standard error of mean ± 2.3

Δ mg/min., mean difference between means of stress and control studies 81.7 ± 23.0 — $P < 0.01$
Δ % change, mean difference between means of stress and control studies 182.5 ± 35.5 — $P < 0.01$
Δ mg./cc, mean difference between means of stress and control studies 4.3 ± 2.9 — $0.2 > P > 0.1$

Analysis of variances reveals no significant difference between stress and control studies with regard to mg/cc (F Test).

* Subjects experienced hunger during these hours.
† Subject in ketosis on arrival.
‡ Values in bold type obtained during stress.

tration of ketone bodies in his blood (20. 8 mg %) at the time of his arrival was a chronically depressed man who was preoccupied with feelings of loneliness and sadness, and bitter ruminations. During an interview in which he was diverted from these thoughts and reassured by the interest of a friendly physician there was a pronounced fall in his blood ketones, accompanied by a significant fall in his rate of urine output. The concentration of glucose, ketones, and chlorides in the urine did not change significantly, however.

Effect of administering water to control subjects

Because hydropenia is probably in itself associated with strong inhibitory influences on diuresis, a second group of subjects during control procedures were given a moderate amount of fluids in the form of 200–250 cc of 0.9% saline intravenously during the interview hour (Table II). In none of these subjects was there a significant difference in the rate of urine output during the interview hour as compared to the average of the first and third hour values, although two of them

TABLE IV

Chloride excretion

	Subject	Chloride excretion (*mg/min.*)					Chloride concentration (*mg/cc*)				
		Before arrival	Observation periods			Δ mg/min.	Before arrival	Observation periods			Δ mg/cc
			1st	2nd	3rd			1st	2nd	3rd	
					Control Studies						
1.	K	11.5	16.0	14.8	13.7	0.0	23.40	27.50	27.50	30.00	+1.20
2.	I		10.0	11.0	10.0	+ 1.0	16.80	18.00	14.30	13.20	− .70
3.	S	1.2	3.2	4.1	4.9	+ 0.5	5.00	7.65	6.20	8.80	−2.00
4.	AB	6.9	3.6	2.7	6.4	− 2.3	5.00	4.60	5.40	5.40	+ .40
5.	I	2.4	6.7	5.4	4.7	− 0.3	4.90	7.25	7.25	7.40	+ .07
6.	U	1.2	2.8	3.0	4.4	− 0.6	7.45	10.80	8.40	11.05	−1.50
7.	P	2.5	4.9	3.1	3.9	− 1.3	7.20	9.05	7.15	7.40	−1.05
8.	Y	4.1	8.3*	10.8*	14 *	+ 0.3	4.40	5.55*	6.35*	8.90*	+ .77
9.	R	4.2	5.7	8.7	9.5	+ 1.1	4.95	2.87	3.90	4.35	+ .29
10.	BL	7.6	7.8	7.3	8.0	− 0.6	9.00	8.95	8.85	9.10	− .17
11.	Z†	3.2	3.2	2.3	1.9	− 0.3	.76	.70	.68	.63	+ .02
12.	AJ	5.8	11.5*	14.4*	22.8*	0.0	8.10	5.70*	6.00*	8.00	+ .05
				19.8*					7.80*		
13.	BC	1.8	2.5	3.0	3.0	+ 0.3	8.75	9.35	10.55	10.40	+ .68
14.	O	1.5	1.4	1.2	0.9	0.0	2.66	3.10	2.58	2.59	− .26

Δ mg/min., mean = − 0.2 Standard error of mean = ± 0.2
Δ mg/cc, mean = − 0.16 Standard error of mean = ± 0.24

Studies during Stress Diuresis‡

1.	AC	2.9	**4.5**	**7.8**	3.0	+ 4.8	2.70	2.20	**4.10**	4.95	− .85
2.	J	3.2	**5.2**	**5.2**	4.5	+ 0.7	7.65	**5.00**	**6.20**	6.50	− .30
3.	AC		5.0	**5.0**	1.5	+15.0		7.40	**3.05**	3.20	−1.28
				18.0					**5.60**		
4.	AN	9.0	7.6	**10.6**	5.3	+ 4.3	8.85	6.75	**5.25**	9.45	−3.35
				7.9					**4.25**		
5.	BH	5.1	**5.9**	**9.0**	**12.2**	+ 6.3	7.68	7.05	**7.32**	**8.35**	+ .78
6.	Y	1.0	**4.5**	**4.5**	4.2	+ 0.3	1.40	**1.60**	**1.60**	2.60	−1.00
7.	Y	3.6	**8.8**	**11.4**	4.2	+ 7.2	3.35	**3.20**	**4.35**	3.75	+ .02
8.	BE	7.0	**11**	**11**	**12.7**	+ 5.7	7.05	**2.60**	**1.75**	**2.50**	−4.77

Δ mg/min., mean = + 5.4 Standard error of mean ± 1.6
Δ mg/cc, mean = − 1.34 Standard error of mean ± 0.65

Δ mg/min., mean difference between means of stress and control studies 5.6 ± 1.6 $P < 0.01$
Δ mg/cc, mean difference between stress and control studies 1.18 ± 0.69 $0.2 > P > 0.1$

* Subjects experienced hunger during these hours.
† Subject was in ketosis on arrival.
‡ Values in bold type obtained during stress.

developed a gradual increase in urine output during the three hour period. Both of these subjects developed moderate hunger during the morning. It is possible that this diuresis may in part represent a "starvation diuresis" as described above. Another subject (R) had a somewhat elevated rate of urine formation at the outset of the study.

Stress Studies

In 11 of the 14 subjects who were exposed to stressful situations there was a significant diuresis (Table I), accompanied by an increase in chloride excretion (Table IV). In subjects who had glycosuria, there was an increase in glucose excretion which was parallel to the increase in water excretion and was not accompanied by significant changes in urine glucose concentration (Table III). This diuresis was nearly always associated with feelings of anxiety and apprehension, although other feelings might be present also. The conflict situation which aroused these feelings was often initiated by the attitude and actions of the interviewer during the initial waiting period, and sometimes, as in Subject BH, could not be allayed during the final period. Stress periods, therefore, were not confined solely to the second hour. The

TABLE V

Ketone excretion (expressed as acetone recovered)

	Subject	Ketone excretion (*mg/min.*)						Ketone concentration (*mg/cc*)				
		Before arrival	Observation periods			Δ mg/min.	Δ %	Before arrival	Observation periods			Δ mg/cc
			1st	2nd	3rd				1st	2nd	3rd	
						Control Studies						
1.	K	.020	.015	.010	.005	0.000	0	.042	.028	.020	.013	−0.005
2.	I		.009	.010	.017	−.003	− 30	.017	.017	.015	.022	− .004
3.	S	.006	.025	.027	.028	−.001	− 3	.023	.057	.040	.044	+ .011
4.	AB	.045	.032	.022	.050	−.019	− 19	.034	.041	.043	.042	+ .0015
5.	I	.020	.049	.040	.038	−.003	− 7	.041	.053	.053	.060	− .004
6.	U	.014	.028	.036	.045	−.001	− 3	.085	.107	.108	.114	− .002
7.	P	.010	.009	.011	.012	+.001	+ 10	.028	.018	.025	.025	+ .004
8.	Y	.019	.066*	.129*	.114*	+.039	+ 43	.019	.043*	.075*	.066*	+ .021
9.	BL	.008	.011	.010	.010	−.001	− 10	.010	.012	.012	.011	+ .0005
10.	Z†	1.930	2.340	1.700	1.650	+.299	+ 15	.461	.510	.520	.540	+ .005
11.	O	.028	.023	.026	.016	+.007	+ 37	.050	.051	.056	.056	+ .003

Δ mg/min., mean = + 0.029 Standard error of mean ± 0.027
Δ % change, mean = + 3 Standard error of mean ± 7
Δ cc/min., mean = + 0.0032 Standard error of mean ± 0.0022

Subjects Having Stress Diuresis

1.	AC	.025	**.011**	**.026**	.009	+.017	+189	.023	**.005**	**.014**	.013	− .005
2.	J	.052	**.160**	**.150**	.200	−.050	− 25	.132	**.157**	**.179**	.195	− .016
3.	AN	.021	.009	**.014**	.016	+.006	+ 50	.021	.008	**.007**	.027	− .008
				.022						**.012**		
4.	BH	.011	.013	**.020**	**.009**	+.001	+ 8	.017	.015	**.017**	**.006**	− .003
5.	Y	.050	**.450**	**.450**	.250	+.200	+ 80	.094	**.158**	**.163**	.156	+ .004
6.	Y	.125	**.278**	**.256**	.143	+.135	+ 94	.117	**.101**	**.097**	.126	− .027
7.	AB	.004	**.016**	**.006**	.021	−.005	− 31	.006	**.008**	**.013**	.020	− .009
8.	BE	.005	**.017**	**.012**	**.064**	+.026	+520	.005	**.004**	**.002**	**.012**	+ .001

Δ mg/min., mean = + 0.041 Standard error of mean ± 0.029
Δ % change, mean = + 111 Standard error of mean ± 64
Δ mg/cc, mean = − 0.0079 Standard error of mean ± 0.0035

Δ mg/min., mean difference between stress and control studies = 0.012 ± 0.040 $1.0 > P > 0.5$
Δ % change, mean difference between stress and control studies 108 ± 64 $0.2 > P > 0.1$
Δ mg/cc, mean difference between stress and control studies 0.0111 ± 0.0041 $0.05 > P > 0.02$

* Subjects experienced hunger during these hours.
† Subject in ketosis on arrival.
‡ Values in bold type obtained during stress.

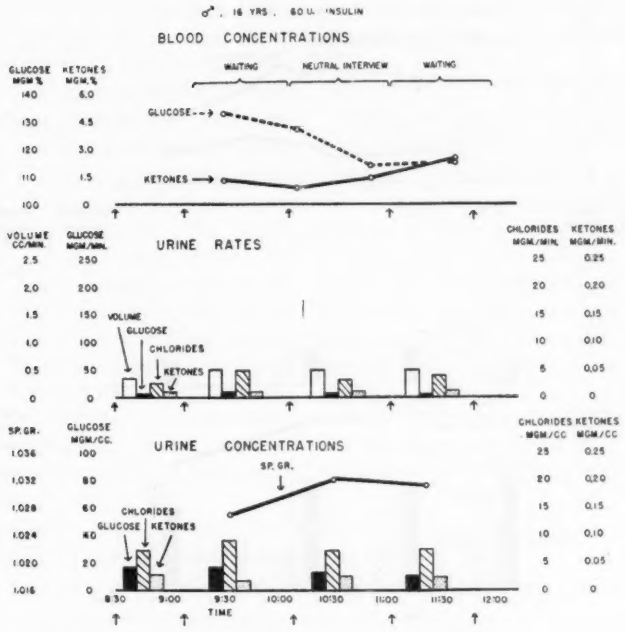

FIG. 1. CONTROL STUDY. URINE GLUCOSE CONCENTRATION 17 MG/CC

Normal rate of excretion of water, chlorides, and ketones; no significant change in concentration of these substances in the urine.

three subjects who developed a significant rise in their blood ketone concentration but had no diuresis showed little evidence of anxiety or apprehension. They were predominantly angry, depressed and resentful. Figure 4 is a diagram of such a study.

Stress diuresis occurred in diabetic subjects even without glycosuria (Figure 5). In such persons the diuresis could not be distinguished from stress diuresis as seen in the non-diabetic (9). It was characterized by an increase in the rate of water excretion, accompanied by a fall in the specific gravity of the urine and in the concentration of chlorides and ketones; but the rise in urine output usually offset the fall in chloride concentration, and resulted in an increased rate of chloride excretion. Accompanying the diuresis there was usually a rise in the blood ketone level, a fall in the blood glucose level, and a decrease in the number of circulating eosinophiles.

When glucose was present in the urine in small or moderate concentrations, the rate of glucose output rose *pari passu* with the rate of water output (Figure 6), but there was no significant change in the concentration of glucose in the urine, and the specific gravity of the urine occasionally fell. When glucose, ketones, and chlorides were initially present in the urine in high concentration, the rate of output of all of these substances also rose in parallel with the rate of water output (Figure 7), again without significant change in their concentrations. It appeared, therefore, that the diuresis was not dependent upon the development of a urine maximally concentrated with regard to glucose or any other substance.

That this diuresis was not dependent upon the level of the blood sugar or upon changes in it was evident from the fact that it occurred in persons with "normal" blood glucose levels (Figure 5) and was most often seen in association with a

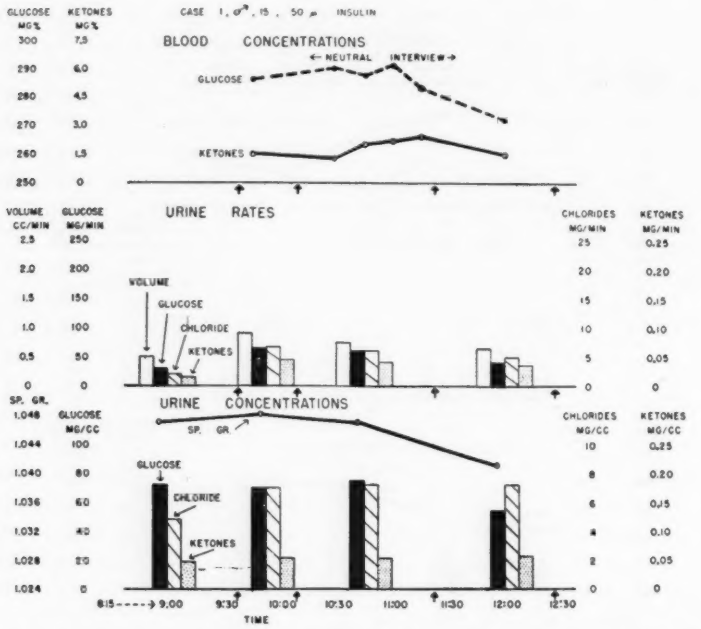

FIG. 2. CONTROL STUDY. HYPERGLYCEMIA AND HEAVY GLYCOSURIA NOT ASSOCIATED WITH POLYURIA

Blood glucose 286 mg%, urine glucose concentration 73.4 mg/cc. Normal rate of output of water, chlorides, and ketones.

falling blood glucose concentration (Figures 6 and 7). On the other hand, it was also seen to occur in persons in whom the blood glucose rose significantly (Figure 8). The subject of this study, who had no glycosuria at the outset of the experiment, developed hyperglycemia, glycosuria, and a slight diuresis after he learned that nothing could be done to prevent him from rapidly losing his eyesight. (This knowledge, which made him predominantly apprehensive and depressed, but did not cause him to feel resentful of the physician or deprived of his support, led to somewhat different feeling states from those aroused by the stresses to which most of the subjects were exposed.) His diuresis probably could not be explained on an osmotic basis, for his urine was at no time maximally concentrated.

Experiments such as that on Subject Q demonstrated that a rise in the blood glucose concentration was not necessarily accompanied by a diuresis. This subject developed a hyperglycemia during a stressful interview, but at the same time developed an oliguria, despite the fact that her urine contained 7.1% glucose and despite the fact that she received 790 cc of fluids during the three hour period. (See below.) A diminution in urine output (cessation of diuresis) also occurred in Subject AB as previously mentioned, during the course of a stress-relieving interview in which his blood sugar level fell. From such studies it appeared that changes in the blood glucose concentration of endogenous origin were not a controlling factor in the development of either a rise or a fall in the urine output of these diabetic subjects, even when their urine was maximally concentrated with regard to glucose.

The magnitude which a stress diuresis may attain was demonstrated by Subject Y. This girl un-

der stress developed a urine output rate of 4.7 cc/min. (282 cc/hr.) and a glucose excretion rate of 490 mg/min. (29.4 gm/hr.). During a four-hour period under stress she excreted 1016 cc of water and 98.25 gm of glucose, whereas on a comparable control day, when her urine contained an equivalent concentration of glucose (90 mg/cc) she excreted only 353 cc. of water and 22.85 gm of glucose during the same period of time. She had not eaten for 14 hours and had presumably been excreting glucose at a relatively high rate prior to the experiment. According to Soskin and Levine (10), the liver of a well fed man of 70 kg contains only 108 gm of glucose as glycogen. Although some of the glucose which this girl excreted probably represents that dissolved in extra-cellular fluid which was mobilized during the period of diuresis, it is a reasonable assumption that a good part of it was derived not only from liver glycogen, but also by gluconeogenesis from protein.

Effect of stress on subjects given fluids

Among the group of nine patients under stress who received fluids at the rate of 200 cc/hr., six developed a significant degree of diuresis (Table II). Two of these subjects, neither of whom was anxious, developed no diuresis, and one who was intensely afraid became oliguric. As mentioned above, this subject developed a hyperglycemia during the stressful interview, but she became oliguric despite this and despite the fact that she received 790 cc of fluids during the three-hour period.

That oliguria may occur during fear or pain has been known for some time. The phenomenon has been studied extensively in animals by Verney

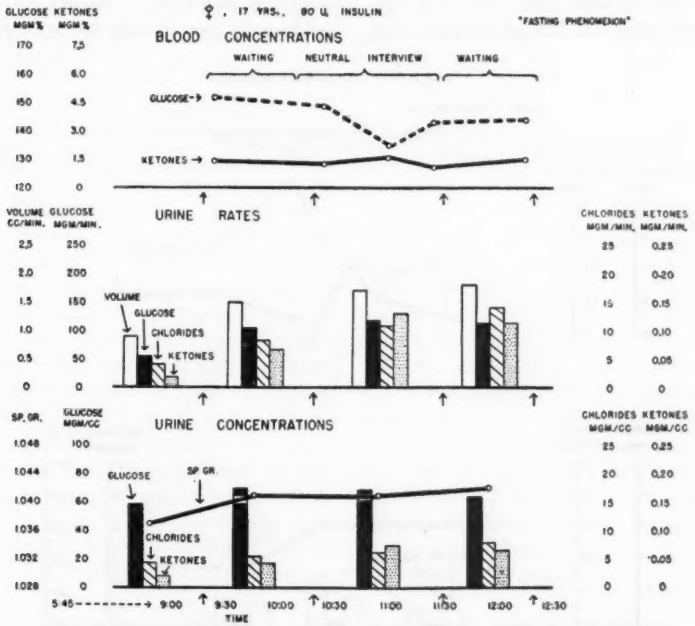

FIG. 3. CONTROL STUDY. "FASTING PHENOMENON" IN A DIABETIC

Gradual rise in the output of water, chlorides, glucose, and ketones associated with increasing hunger and tension. Slight fall in blood glucose, and no significant change in urine glucose concentration.

(11), and in man it has been described by Wolf (12) and Kelsall (13). It appears to be the physiological response which is the opposite of the diuresis of stress, and to be induced by circumstances which imply a different sort of threat to the subject from those which elicit a diuresis. The relation between "stress diuresis" and "stress oliguria" seems to be analogous to the relation between the vascular hypertension and hypotension which may occur in stressful situations. That is to say, just as an individual who may develop a marked rise in blood pressure when angry, may on the other hand develop acute hypotension and faint at the sight of blood, so the same individual may develop diuresis under one set of circumstances, and oliguria under another. (See discussion of Subject AB, below.)

Effect of stress on persons ingesting concentrated glucose solution

Two other subjects were given 500 cc of 50% glucose by mouth after initial blood and urine samples had been obtained (Table VI). Control and stress studies were made upon each subject. The procedures were physically identical, and were separated by an interval of one month during which there was no significant change in the patient's diet, insulin intake, average daily activity, or state of general health. Subjects had received their last insulin injection 24 hours before the procedure. The control studies were made on days when the subjects were feeling relatively cheerful and secure, and when their personal problems were not especially pressing; during the neutral interviews they were lightly diverted by friendly talk with a physician whom they knew and liked, including a discussion of topics and situations which they had associated with hypoglycemia ("insulin reactions"). The stress studies, on the other hand, were made on days when the subjects were feeling depressed, resentful, anxious, and unloved, and when their personal problems were acutely obtruding themselves. Subject V, for example, had recently been passing through a period of con-

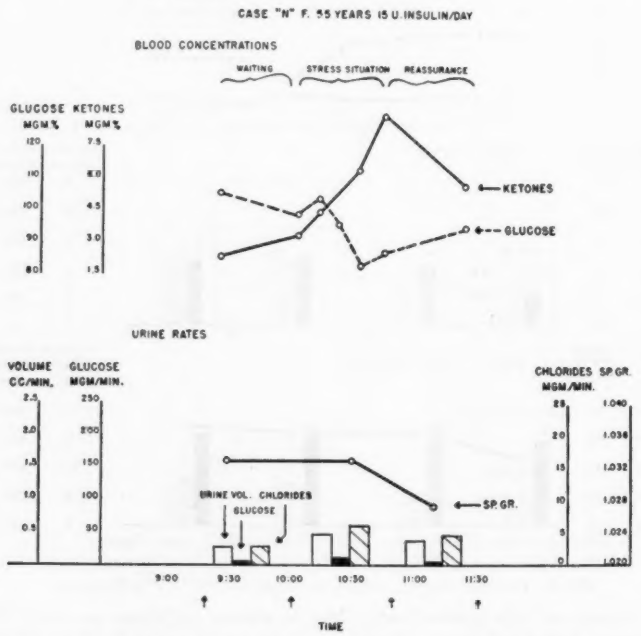

FIG. 4. STRESS STUDY. RISE IN BLOOD KETONES OCCURRING DURING STRESS, NOT ACCOMPANIED BY DIURESIS

TABLE VI

Effect of ingesting 500 cc of 50% glucose solution

	On arrival	Glucose P.O.	Observation periods* 1st	2nd	3rd	4th	Total excreted	Increment over baseline†
Subject V								
Control Studies								
Volume, cc/min.	1.8		1.2		5.5	5.1	812 cc	+445 cc
Glucose excr., mg/min.	0		0		365.	645.	73.2 gm	+ 73.2 gm
Chloride excr., mg/min.	11.0		4.1		11.7	17.5	2.36 gm	+ 0.12 gm
Ketone excr., mg/min.	.034	↓	.028		.017	.017	2.56 mg	− 4.26 mg
Stress Studies								
Volume, cc/min.	0.4		**2.2**	**7.7**	**5.2**	**7.2**	1058 cc	+976 cc
Glucose excr., mg/min.	8		**118**	**279**	**350**	**511**	61.34 gm	+ 59.71 gm
Chloride excr., mg/min.	3.2		**9.3**	**16.3**	**16.2**	**25.2**	3.28 gm	+ 2.626 gm
Ketone excr., mg/min.	.025	↓	**.046**	**.093**	**.052**	**.072**	13.39 mg	+ 8.29 mg
Subject K								
Control Studies								
Volume, cc/min.	0.5		0.8 0.6	0.4	0.7	1.8	180.3 cc	+ 71 cc
Glucose excr., mg/min.	0		0 0	7.7	25.9	112	6.36 gm	+ 6.36 gm
Chloride excr., mg/min.	4.4		9.0 6.6	3.2	2.2	4.2	0.987 gm	+ .022 gm
Ketone excr., mg/min.	.010	↓	.007 .009	.014	.014	.057	4.220 mg	+ 2.03 mg
Stress Studies								
Volume, cc/min.	2.0		**2.4**	**4.35**	**4.33**	**4.72**	845 cc	+407 cc
Glucose excr., mg/min.	135		**177**	**336**	**354**	**342**	64.0 gm	+ 29.6 gm
Chloride excr., mg/min.	6.6		**10.0**	**15.8**	**13.5**	**14.8**	2.919 gm	+ 1.479 gm
Ketone excr., mg/min.	0.24	↓	**0.43**	**0.42**	**0.43**	**0.46**	96.4 mg	+ 43.8 mg

* Values in bold type obtained during stress.

† "Increment over baseline" represents the actual amount of each substance which was excreted during the four hour period after the ingestion of glucose, over and above the amount which would have been excreted during the same period if the subject had continued to excrete at the rate which obtained during the initial control period.

flict with her alcoholic husband and her domineering mother-in-law (4), while Subject K was unemployed, discouraged by rude rebuffs when he attempted to find a job, and brooding over the fact that he would be unable to buy his children any toys for Christmas. During the stress interviews the physician assumed a cool and detached manner, and pressed the discussion of these unpleasant situations depite the subject's desire to avoid them.

A diuresis ensued in each of these experiments, although in Subject K the diuresis during the control procedure was delayed and small in magnitude. In each of these subjects the diuresis during stress was much greater in terms of loss of water, chlorides, and ketones. The two studies made on Subject V are diagrammed in Figures 9 and 10, and have been discussed in another paper (4). Although she excreted almost the same amount of glucose on each occasion, she lost 39% more water, and 57% more chlorides during the stress procedure. Her chloride excretion rose to 25.2 mg/min., which is the equivalent of a loss of 1.5 gm/hr. or 36.3 gm in 24 hours. The incremental excretion of glucose was actually greater during the control procedure (73.2 gm) than during the stress procedure (59.7 gm), but during the control study the increment of water excretion (445 cc) was less than half as much as that during the stress study (976 cc). The diuresis on the control day was associated with a negligible increment of chloride loss (0.12 gm), while that on the stress day was associated with a large increment of chloride loss (2.626 gm). On the control day the ingestion of glucose led to a significant decrease in ketone excretion, whereas on the stress day it was followed by a slightly increased ketonuria.

Closely similar changes were observed in the two studies on Subject K.

DISCUSSION

It seems to be clear from these observations that in the evaluation of diuresis in diabetes it is

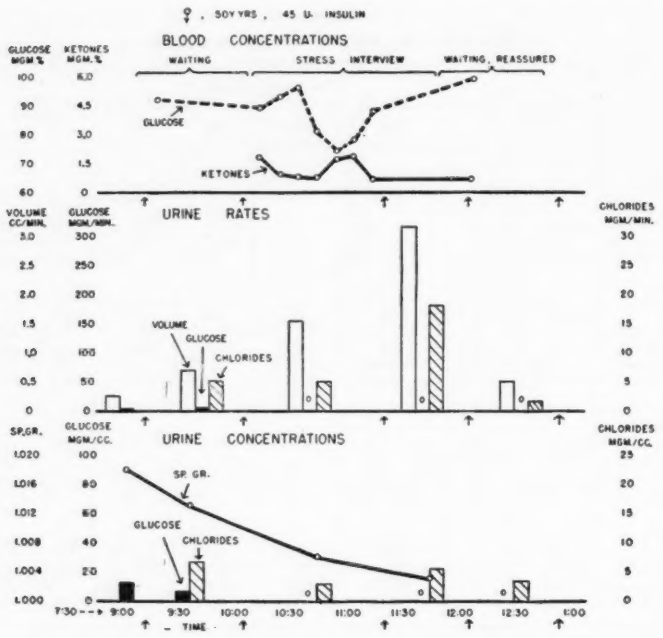

FIG. 5. STRESS STUDY. DIURESIS OCCURRING IN THE ABSENCE OF GLYCOSURIA

Rate of chloride excretion rises in parallel with rise in rate of water excretion, although specific gravity of urine and concentration of chlorides falls.

necessary to consider factors other than the concentration of glucose in blood and urine, since diuresis involving loss of water and chlorides may occur independently of changes in glucose and ketone excretion. In this respect diabetics behave in a fashion similar to non-diabetic individuals.

The occurrence of a "diuresis of stress" in persons who do not have diabetes mellitus appears to have little or no adverse effect upon their health; but when this phenomenon occurs in a diabetic it may have serious consequences. The loss of glucose, water, and chlorides which may occur under circumstances of stress may cause a severe degree of dehydration if it continues. In an earlier communication it was pointed out that stressful situations may also lead to a direct augmentation of the blood ketone concentration in both diabetic and non-diabetic persons(8). A simple elevation of the ketone concentration in the blood does not appear to be toxic to either diabetics or non-diabetics; but when a rise in ketonemia is accompanied by diuresis and dehydration, it may be a potent factor in the development of diabetic acidosis and coma. It is probable that this reactivity of the diabetic to the stresses of daily life explains many of the hitherto obscure fluctuations in the course of diabetes. In extended clinical studies published elsewhere (4–7) it has been observed repeatedly that not only episodes of ketosis and coma, but also changes in insulin requirement of short or long duration, occurring in diabetics of all grades of severity, show an intimate temporal correlation with the significant events in their lives. In adolescents many instances of ketosis have been seen to occur within a relatively few hours after a severe conflict with parents, quite in the absence of infections or of significant changes in their diet or insulin intake. Dolger (14) states that he observed an adolescent girl on a hospital ward who, in a conflict situation, within six hours developed

severe ketosis requiring over 400 units of insulin for its treatment.

The fact that the ingestion of large amounts of glucose and water during a period of stress serves to increase the magnitude of the diuresis may explain another frequently observed phenomenon. Clinicians have long noted that persons with diabetes often are admitted to the hospital in coma after an "eating spree." For a long time it was felt that the ingestion of food was the cause of the ketosis, but Mirsky and others (15, 16) have conclusively demonstrated that a simple ingestion of food cannot cause ketosis. The present study indicates that during periods of calm and security, in the absence of stressful situations, a diabetic person may tolerate the ingestion of large amounts of glucose and water quite well; but that the ingestion of similar amounts of glucose and water in the face of an established stress diuresis increases the loss of chloride and has a potentially deleterious effect. Moreover, the investigations cited above (4, 5) have indicated that diabetic persons tend to respond to stress with an increased desire to eat. It appears, therefore, that diabetic persons do not "eat themselves into coma," but rather that diabetic persons when subjected to stress respond with ketonemia, diuresis and an increased ingestion of food, and that under these circumstances

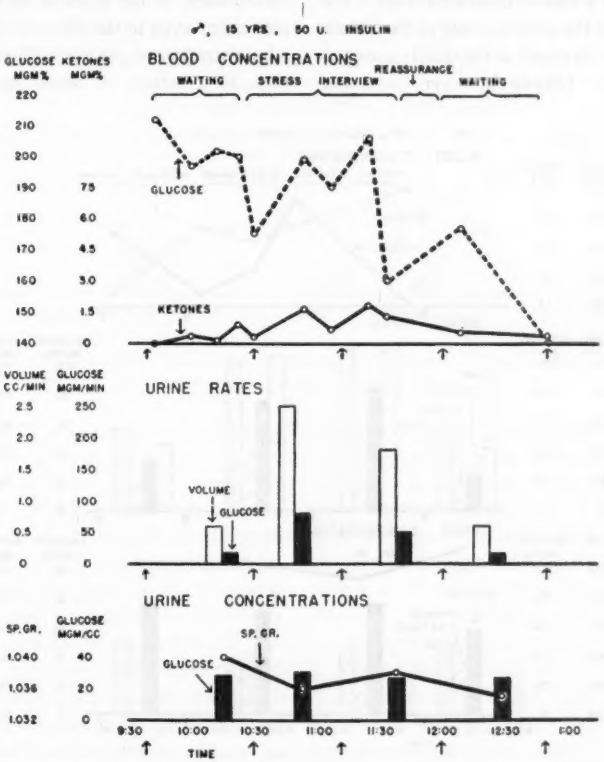

FIG. 6. STRESS STUDY. DIURESIS OCCURRING IN A SUBJECT WITH MODERATE GLYCOSURIA

Rate of glucose excretion rises in parallel with rate of water excretion; concentration of glucose in urine does not change significantly. Diuresis accompanied by large fall in blood glucose associated with marked fluctuations in the level.

the ingestion of large amounts of water and concentrated carbohydrate may aggravate the diuresis.

The fact that a diuresis of stress is associated with an increased excretion of chloride may have an important bearing upon the loss of fixed base which occurs in diabetic acidosis. In 1933 Atchley and associates (17) observed that when diabetic subjects on a metabolism ward were allowed to develop ketosis because of lack of insulin, the early stages of the ketosis were accompanied by a loss of chloride as well as sodium and potassium in the urine. Such a chloruresis would appear to be "contraindicated" from a homeostatic point of view, inasmuch as a loss of chloride entails a loss of fixed base, while the acidotic state of the ketotic person appears to demand a maximal conservation of fixed base. Others, however, have confirmed this finding (18, 19) and it has now been established that chloruresis probably accounts for a large part of the loss of fixed base which occurs during the early stages of ketosis (18, 20). Atchley and co-workers attributed this loss of chloride to the heavy glycosuria. Although experimental attempts to cause chloride depletion in diabetics by giving them glucose have not always been successful (21), recent studies indicate that the administration of glucose to diabetics leads to a significant chloride loss (20). The present studies indicate that some of the loss of chloride which occurs in a diabetic may be a concomitant of the diuresis of stress, and that it may occur even in the absence of glycosuria. The administration of glucose without insulin to a diabetic in a setting of stress appears to cause a

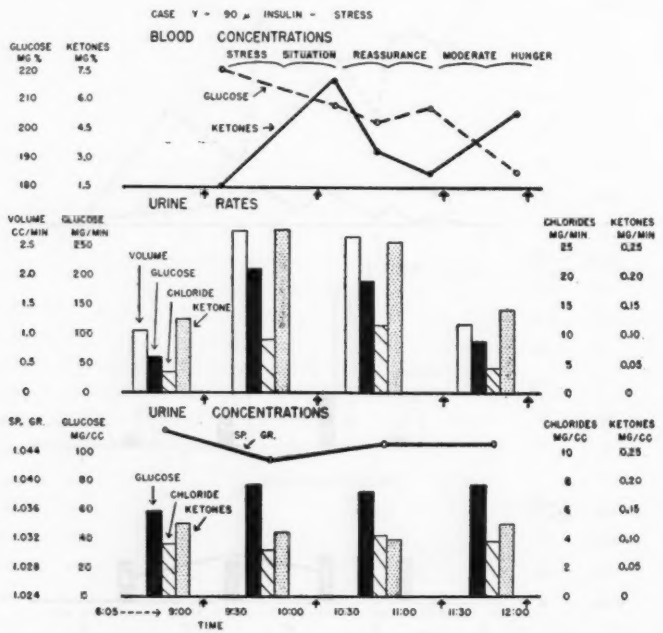

FIG. 7. STRESS STUDY. TYPICAL STRESS EFFECT ON A DIABETIC WITH HYPERGLYCEMIA AND A HEAVY GLYCOSURIA

Diuresis, characterized by rise in rate of excretion of water, chlorides, glucose, and ketones, but not associated with significant change in the concentration of these substances in the urine. Rise in blood ketones associated with a fall in blood glucose.

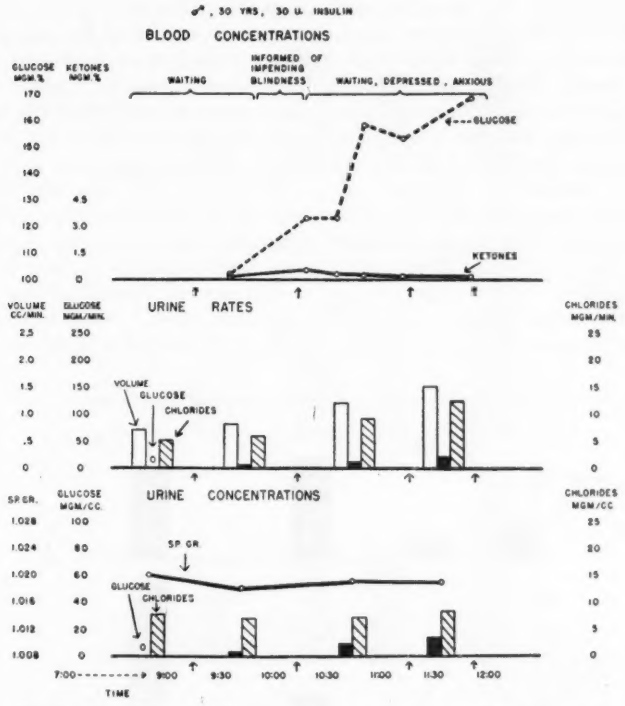

FIG. 8. STRESS STUDY. DIABETIC SUBJECT DEVELOPING HYPERGLYCEMIA, GLYCOSURIA, AND DIURESIS DURING STRESS

greater loss of chloride than occurs when glucose is administered in the absence of stress.

From the present studies it seems safe to conclude that the rate of urine output in diabetic subjects may bear no direct relation to the level of the blood glucose. Many of these subjects developed a ketonemia and a massive diuresis at a time when their blood glucose level was falling steadily, while others with an elevated blood glucose and a heavy glycosuria were obviously in good equilibrium during a morning in which they were under no special stress. Thus it would appear that despite the common clinical reliance on the level of fasting blood sugar as an indicator of "diabetic control," the rate of urine formation is in general a much more reliable reflector of the immediate degree of equilibrium of the diabetic person, if it is evaluated in the light of the general clinical state of the patient and the presence or absence of glycosuria and ketonuria (and, of course, with due consideration of the amount of food or fluids recently ingested).

In order to explain the lack of correspondence between fluid output and the concentration of glucose in the blood and urine, it is necessary to consider the renal mechanism involved. It has been customary to regard the glomerular filtration rate (GF) and the maximal rate of tubular reabsorption of glucose (Glucose Tm) as relatively unvarying in each individual (22–25). Some diabetic persons have been found to have an elevated glucose Tm (26, 27), but this, too, has not been thought to change greatly. The present observations suggest that one or both of these values may change under stress. In fact it is a reasonable assumption that not only the glomerular filtration

and the glucose reabsorption, but the entire renal hemodynamics and all of the reabsorptive capacities of the renal tubule may change under the influence of stress. Smith (28), G. A. Wolf (12), and Stewart Wolf and associates (29), have shown that changes in glomerular filtration, in the filtration fraction, and in the renal blood flow, may occur in response to stressful life experiences, although the changes in glomerular filtration which they described were not of the magnitude which we suggest may have occurred in these studies. Conn and his co-workers (30) have indicated that ACTH depresses the tubular reabsorptive capacity for glucose, while Ingbar and his associates (31) have shown that ACTH and cortisone cause an increase in renal blood flow and glomerular filtration. It has been mentioned that the response to stress of the patients in the present study was often accompanied by a significant fall in the eosinophile count, the details of which will be described elsewhere. It is reasonable to assume that adrenal corticosteroids were elaborated during these procedures. It is of further interest that Verney (11) has shown that the secretion of posterior-pituitary anti-diuretic hormone is under the control of the central nervous system and may be affected by "emotional stress." Some such mechanism must probably be invoked to explain the changes in wa-

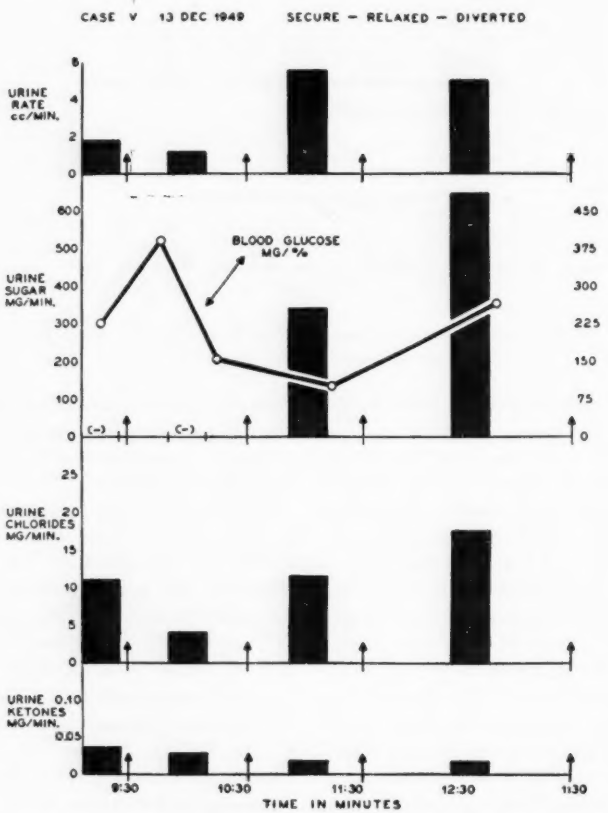

FIG. 9. CONTROL STUDY. EFFECT OF INGESTION OF 500 CC OF 50% GLUCOSE SOLUTION IN THE ABSENCE OF SIGNIFICANT STRESS

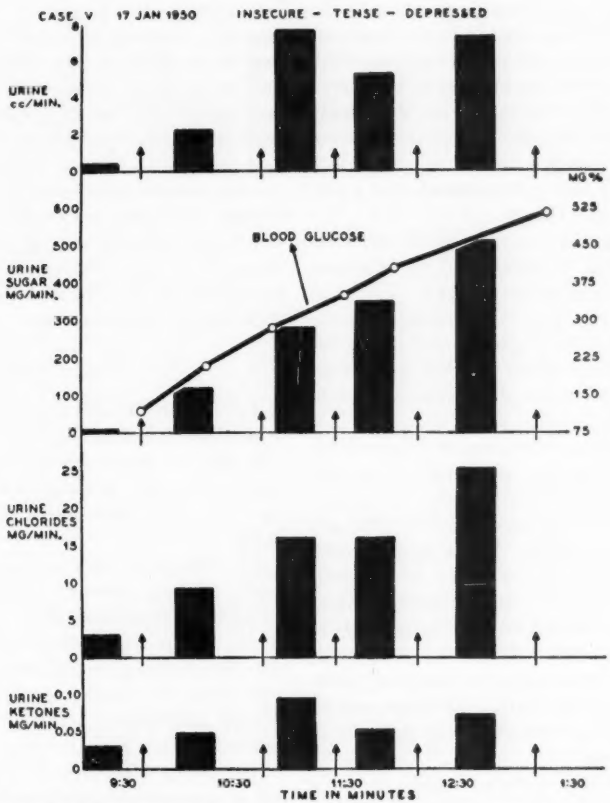

FIG. 10. STRESS STUDY. EFFECT OF INGESTION OF 500 CC OF 50% GLUCOSE SOLUTION IN A SETTING OF STRESS

ter excretion which occurred in both these and the non-diabetic persons previously studied, since present evidence indicates that changes in water excretion cannot be explained upon the basis of changes in glomerular filtration (*32*).

With regard to the connotation of the commonly used expression "emotional stress," it may be said that situations such as these persons faced quite commonly arouse strong emotions, but it is unnecessary to consider that these emotions are the "cause" of the physiological changes which were observed. It appears, rather, that the situation—the words and actions of the interviewer—was the stimulus; that the patient's interpretation of these words and actions, made on the basis of his past experience of which he was partly conscious and partly unconscious, endowed them with a threatening meaning for him, and converted them into a "stress"; and that he responded to this stress with a change in feeling state, a change in behavior, and a change in many aspects of his metabolism. The emotion and the bodily change, therefore, were both a part of the response to stress, and it is not profitable to consider that either caused the other. Despite this lack of causal relationship between the two it is of interest that there did appear to be a high degree of correlation between certain feeling states and specific metabolic changes. Since the

significance of the experimental situation to the individual concerned appeared to be the determining factor with regard to the type of response which occurred, and since the way in which he considered this situation to affect his welfare would naturally be reflected in his attitude, one would expect a fairly high correlation between feeling states which are appropriate to this or that attitude and a particular type of bodily change.

Stress diuresis, for example, most often occurred in a setting of anxiety and apprehension, but intense fear was associated with oliguria, as in Subject Q. Subject AB (Table I, Stress Study) was anxious and apprehensive during the initial hour of observation, and had a diuresis; during the second hour his anxiety turned to great fear, and he became oliguric; during the final hour his urine output returned to its original level. A rise in blood ketones was usually associated with feelings of resentment, hostility, and depression, and of being deprived of the friendship and support of the physician. When mixed feeling states were present, several types of metabolic change might occur also. Measurements of these emotional changes cannot be made as precisely as need be, but conscious and unconscious attitudes and feelings can nevertheless be reasonably satisfactorily assessed with suitable experience and recourse to the methods outlined in the beginning of this communication.

The reasons why a diuresis should occur in a diabetic person in a setting of stress are not at present clear. Usually bodily reactions which develop in situations of stress appear to have an adaptive value for meeting specific threats to the homeostatic integrity of the organism; but the specific threat which they are designed to meet may not actually be a part of the stress to which the subject is now exposed (33). In line with this thought, it is well known that the normal individual adapts to starvation with metabolic changes which involve the preferential use of fat rather than carbohydrate as the fuel for muscular work. This shift is accompanied by a rise in the blood ketones and a diuresis (9, 34), and its development may be accelerated and augmented by exposing the individual to various forms of non-specific stress (9). The fact that the diabetic metabolism has many qualitative similarities to the metabolism of starvation (6), the psychological evidence that diabetes often occurs in persons who have suffered repeated emotional deprivations and who react psychologically as if they were "starved" (4–6), and the similarity of the stress reaction in the diabetic to the reaction of a non-diabetic person to early starvation (9), all have led us to conjecture that perhaps the diabetic individual reacts to the specific stressful situations with which he is confronted "as if they represented threats of starvation," and responds with an exaggeration of the metabolic changes which would be appropriate to actual starvation. The diuresis of stress appears to be a part of this response.

CONCLUSIONS

(*1*) In persons with diabetes mellitus, exposure to stressful life situations may lead to a diuresis. This diuresis is characterized by a 200–500% increase in the rate of water excretion, accompanied by a rise in the excretion of chlorides and ketone bodies.

(*2*) The stress diuresis which occurs in aglycosuric diabetic persons is similar to that observed in non-diabetic individuals.

(*3*) When stress diuresis occurs in diabetic persons who are glycosuric, the rate of glucose excretion rises in parallel with the rate of water excretion, and there is no major change in the glucose concentration of the urine.

(*4*) Stress diuresis is not dependent upon osmotic changes associated with the excretion of glucose in the urine, or upon changes in the concentration of glucose in the blood.

(*5*) A stress diuresis may lead to a rapid loss of large amounts of water, glucose, and chlorides, and may be an important factor in the development of diabetic acidosis and coma.

(*6*) The ingestion of concentrated glucose solutions is well tolerated by diabetic persons in the absence of stress; but in a setting of stress the ingestion of concentrated glucose solutions may accentuate the loss of water and chlorides.

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THE USE OF ARTIFICIAL RESPIRATION IN PULMONARY EMPHYSEMA ACCOMPANIED BY HIGH CARBON DIOXIDE LEVELS [1]

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A syndrome resembling carbon dioxide narcosis, which may be precipitated by the administration of oxygen to patients with advanced emphysema, has attracted increasing attention in recent years, although few reports on this subject have appeared in the literature (1–7). A similar response to oxygen has been described by Marshall and Rosenfeld (8) in experimental animals anesthetized with barbiturates or morphine. Marshall and Rosenfeld's analysis provides a basis for understanding the phenomenon in man; in the presence of an increased level of carbon dioxide in the alveolar air and arterial blood, the respiratory center becomes adjusted to the high carbon dioxide pressure, so that carbon dioxide no longer dominates the chemical control of respiration. Such a loss of sensitivity to carbon dioxide in patients with advanced emphysema was demonstrated in 1920 by Scott (9). In the presence of high levels of carbon dioxide and diminished responsiveness of the respiratory center, the principal stimulus to breathing arises from the effects of hypoxia on the carotid body and aortic chemoreceptors. When the stimulus is removed by relief of the hypoxia through oxygen administration, the driving mechanism is weakened or absent, and pulmonary ventilation falls.

The reduction of ventilation induced by oxygen administration results in further accumulation of carbon dioxide in the body, and since excessive levels of carbon dioxide not only fail to stimulate breathing but actually depress it (10–12), the disease may proceed to a fatal outcome due partially at least to the depressing and narcotic effects of carbon dioxide.

The dilemma presented when an obviously cyanotic patient becomes apneic or almost apneic and comatose when given oxygen has been considered a difficult therapeutic problem. Various recommendations have included the use of carbon dioxide-oxygen mixtures, and, more commonly, the use of relatively low concentrations of oxygen, in the range of 30 to 40 per cent. Evidence will be presented in this report that the prime need of such patients is the elimination of carbon dioxide by increased lung ventilation, and that restored sensitivity to carbon dioxide may be, at least in some patients, of lasting benefit.

Consideration of the use of mechanical aids to respiration in emphysema patients raises the question of the choice of method. There is no ideal method of treatment at this stage of the disease, as such patients are critically ill and understandably intolerant of any procedure which disturbs their already labored breathing. Since there is usually an element of airway obstruction in emphysema, it would be theoretically desirable to apply pressure to assist expiration and inspiration with the timing determined by and precisely dependent on the patient's own respiratory efforts. Such an apparatus, even if available, would have to be applied by a mask or hood; the difficulties encountered in obtaining the necessary tightness of fit for prolonged periods in such patients are often insurmountable.

An alternative method is to enclose the patient's body in a respirator, for the use of negative pressure around the body to assist inspiration and positive pressure around the body to assist expiration. The advantages of this method are that the head is free and that a wide range of pressures can be attained. The disadvantages are that the respirator attempts to set the rhythm of breathing, the patient must be recumbent, and nursing care is impeded. A theoretic advantage of the respirator is that a continuous positive pressure could be used within the tank, reducing the lung volume to a point where the inspiratory muscles could work more effectively.

This report describes the use of the body res-

[1] Aided by a contract between the Medical Division, Chemical Corps, U. S. Army, and Harvard University.

pirator in two patients with severe emphysema accompanied by high carbon dioxide levels. A report is made at present for two reasons: *1*) an adequate number of cases for studies of this type is difficult to obtain; *2*) although results were disappointing in one patient, the effects on the other were so dramatic that recommendation for further trial of the therapeutic procedure seemed warranted.

MATERIAL AND METHODS

Patient 1 (N. G.) was a 58 year old white male candyworker, admitted to the Peter Bent Brigham Hospital on February 1, 1950, with a history of persistent headache, somnolence, dyspnea on exertion, and ankle edema. His first admission was in 1947, with similar complaints. The patient was at that time found to have marked emphysema with secondary polycythemia; he had an arterial oxygen saturation of 82 per cent, and mild cor pulmonale. Cardiac output was 7.3 liters per minute, and the findings suggested early right heart failure. Pneumotachograms at that time showed a pattern of respiration characteristic of marked emphysema, with a minute volume of 6.9 liters per minute. Investigations to determine the cause of the somnolence were not rewarding, and it was concluded that this symptom was due to cerebral hypoxia. An isolated measurement of carbon dioxide serum level was 29 mM per liter.

Since 1947 the patient continued to work, with frequent phlebotomies to control headaches, but his symptoms increased in severity before this admission. When in the outpatient department he had marked somnolence and mental confusion; he was very cyanotic with slow shallow respirations and 2 + ankle edema.

Family history and past history were non-contributory except for respiratory symptoms. There had been marked dyspnea on exertion for some years, accompanied by a chronic cough productive of scanty sputum. The patient worked in a coal mine for two years just before World War I.

Findings on admission: The patient was cyanosed, orthopneic, and confused. The temperature was 99.5, pulse 94, respirations 16, and blood pressure 140/84. Respiration was shallow, with a prolonged expiratory phase. The chest had an emphysematous appearance, with increased anteroposterior diameters. To percussion, the chest was hyperresonant throughout, with no areas of dullness; the diaphragms appeared to be low. There were a few moist râles in both lungs, with occasional musical expiratory rhonchi. No clubbing of the fingers was present. There was some distension of neck veins, and 2 + swelling of the ankles.

Roentgenograms of the chest showed smooth low diaphragms which moved equally. Lung markings were increased, and costophrenic angles were clear.

The hematocrit was 49.2 per cent on admission, and was reduced to 45 per cent after phlebotomy.

It was observed that when oxygen was given, the patient's condition deteriorated and there was marked reduction in ventilation.

Blood chemistry figures were: blood urea nitrogen 17 to 39 mg. per cent, protein 6.1 gm. per cent, sugar 71 to 93 mg. per cent, serum carbon dioxide 35.3 to 41.4 mM per liter, chlorides 83 to 91 mEq, and total base 139 mEq.

Patient 2 (J. M.) was a 55 year old white male clerical worker, admitted to the Veterans Administration Hospital, West Roxbury, June 20, 1950, with a history of shortness of breath and wheezing.

This was his fourth admission, the first being in September, 1946, with a diagnosis of emphysema, bronchiectasis, and malnutrition; the second admission was in August, 1948, with a diagnosis of emphysema and right hydronephrosis; and the third was in January, 1949, with a diagnosis of emphysema and a conversion reaction. The presence of emphysema had been recognized for six years. There was increasing dyspnea on exertion, and recently the patient could not take three steps on the level without gasping for breath. He was obliged to give up work. The dyspnea varied greatly from time to time. There had been a chronic cough with whitish sputum for years and intermittent wheezing. Occasional ankle edema had been present. For three months prior to this admission there was fatigue and increasing weakness.

Past illnesses included influenza in World War I, but nothing else of significance.

Findings on admission: The patient was cyanosed, dyspneic, and apprehensive. The blood pressure was 110/60, and the pulse 96. The chest showed increase of the anterior-posterior diameter, with poor movement. Breath sounds were distant, and the lung fields hyperresonant. No added sounds were heard.

X-ray of the chest showed the diaphragm low and the lung fields emphysematous.

The hematocrit was 61 per cent and the patient had 17 gm. per cent hemoglobin. The serology was negative. A white count showed 9,200 per cubic millimeter, with 78 per cent polymorphs. Chemical examination of the blood gave: nonprotein nitrogen 21 mg. per cent, glucose 97 mg. per cent, chlorides 82 mEq, serum carbon dioxide 40 mM per liter. The electrocardiogram showed right ventricular strain. A repeat white blood count gave 8,200 per cubic millimeter, with 63 per cent polymorphs.

Respiratory ventilation measurements were made by means of a Benedict-Roth type recording spirometer. Pneumotachograms were recorded by the instrument described by Silverman and Whittenberger (13). Residual air was measured by a helium dilution method (14). Alveolar air carbon dioxide was measured by an Engelhard thermoconductivity meter after collection of alveolar air with an automatic sampling device (15). Blood gas partial pressures were measured in arterial samples by the microbubble technique of Riley, Proemmel, and Franke (16). On some samples the Van Slyke-Neill technique was used for oxygen content and capacity and carbon dioxide content (17).

TABLE I

Analysis of arterial blood samples before respirator treatment (N. G., February 7, 1950)

Conditions	pO_2	pCO_2	Serum CO_2	pH	O_2 content	O_2 capacity	O_2 saturation	CO_2 content
	mm. Hg	*mm. Hg*	*vol. %*		*vol. %*	*vol. %*	*%*	*vol. %*
Room air	39	67	94.9	7.41	clotted	—	73.0	—
100% oxygen, 7 minutes	118	76	103.0	7.38	16.69*	16.74	99.7	85.1

Hematocrit 41%.
* Not corrected for dissolved oxygen.

RESULTS

The blood gas data in patient N. G. when he was semicomatose, before respirator treatment, are given in Table I. The arterial pO_2 was only 39 mm. Hg, resulting in a saturation value of 73 per cent, at a pH of 7.41 and pCO_2 of 67 mm. Hg. Although the pCO_2 was markedly elevated, the carbon dioxide content of serum was correspondingly increased, giving a pH of 7.41.

TABLE II

Ventilation and pCO_2 responses to oxygen before respirator treatment (N. G.)

Date	Ventilation		Alveolar pCO_2*	
	On room air	On oxygen	On room air	On oxygen
1950	*liters per minute*		*mm. Hg*	
Feb. 4	7.4	5.2	66	77
Feb. 6	7.3	5.0	63	87
Feb. 7	7.8	5.4	66	78

* Normal pCO_2 at sea level is 39 ± 2 mm. Hg.

The same table illustrates the deleterious effects of oxygen in such a patient. After seven minutes of breathing cylinder oxygen (open circuit) the pCO_2 had increased to 76 mm. Hg. The carbon dioxide content had risen somewhat and the pH was down to 7.38. The pO_2 was, at 118 mm. Hg, sufficient to saturate hemoglobin to the normal value and thus to relieve cyanosis.

The ventilation volumes and alveolar carbon dioxide during the pretreatment phase and during oxygen administration are given in Table II.

Ventilation and alveolar pCO_2 data during and after respirator treatment are summarized in Table III. The first measurements were made approximately 18 hours after respirator treatment was started; the high pCO_2 in spite of the large minute volume can probably be explained by the fact that the patient "fought" the respirator, breathing rapidly and shallowly on his own (rate 47 per minute), thus having a relatively low rate of effective alveolar ventilation.

On the second day of treatment there was still

TABLE III

Lung ventilation and alveolar carbon dioxide during and after respirator treatment (N. G.)

Date	Conditions	Minute volume		Alveolar pCO_2	
		Room air	100% O_2	Room air	100% O_2
1950		*liters*	*liters*	*mm. Hg*	*mm. Hg*
Feb. 12	Entered respirator	—	—	—	—
Feb. 13	In respirator	14.6	—	55	100*
Feb. 14	In respirator	13.5	—	46	52
Feb. 15	In respirator	—	—	41	—
Feb. 15	Respirator open during observation	9.34	7.7	46	46
Feb. 16	Respirator treatment terminated 9:00 a.m.	—	—	—	—
Feb. 16	Out of respirator	9.1	—	47	59
Feb. 17	Out of respirator	—	12.42	39	41
Feb. 18	Out of respirator	13.7	—	38	—
Feb. 19	Out of respirator	—	—	37	—
Feb. 20	Out of respirator	—	—	39	—
Feb. 21	Out of respirator	17.8	—	38	30
Mar. 1	Out of respirator	18.9	17.85	33	—
Mar. 15	Out of respirator	13.4	15.0	31	—

* Closed glottis and did not co-ordinate with respirator.

TABLE IV

Arterial blood gases at five days and at 11½ months following re-acclimatization (N. G.)

Date	Conditions	pCO_2	pO_2	Serum CO_2	pH	O_2 content	O_2 capacity	O_2 saturation	CO_2 content
		mm. Hg	*mm. Hg*	*vol. %*		*vol. %*	*vol. %*	*%*	*vol. %*
2/21/50	Room air	37	67	66.42	7.51	14.65	15.66	92.1	55.12
2/21/50	On 95% O_2 for 31 min.	31	—	62.47	7.54	17.41	15.66	109.6	51.77
1/25/51	Room air	37	76	64.48	7.50	18.97	20.28	92.4	53.23

depression of breathing when high oxygen was given, as indicated by the rise in pCO_2 from 46 to 52 mm. Hg. Subsequent values for ventilation were high, and were accompanied by reasonably low alveolar pCO_2. The high level of carbon dioxide (47 mm. Hg) at the conclusion of respirator treatment was probably due to a sedative drug inadvertently given.

Analysis of arterial blood at intervals of five days and 11½ months after respirator treatment, with the patient breathing room air or a high oxygen mixture, gave the results found in Table IV. On room air the pCO_2 was slightly below normal on both occasions, and the arterial oxygen saturations were 92 per cent. Oxygen administration no longer led to an elevation of pCO_2; in fact, ventilation was stimulated during oxygen administration, possibly due to the patient's enthusiasm to demonstrate his ability. It was difficult to obtain "steady state" values at any time because of the patient's enthusiasm; the gas exchange ratio at the time of the last observation was 1.00, confirming the impression of overbreathing.

Table V shows the ventilatory response of the patient when a mixture of carbon dioxide and oxygen was administered. Whereas the carbon dioxide mixture depressed ventilation before respirator treatment, the normal augmentatory response to carbon dioxide was subsequently restored.

Owing to poor co-operation by the patient, the vital capacity and residual air measurements obtained before artificial respiration therapy were regarded as valueless. A maximum breathing capacity of 36 liters per minute was obtained; this measurement also was impaired by the lack of co-operation. Following therapy, vital capacity was measured as 1.86 liters, the residual air 3.66 liters, and maximum breathing capacity 60 liters per minute.

TABLE V

Response of ventilation to 5.25% carbon dioxide and 94.75% oxygen (N. G.)

Date	Conditions	Minute volume on		Remarks
		Room air	CO_2 mixture	
1950		*liters per minute*		
Feb. 6	Pre-respirator	6.9	5.5	Six minutes of inhalation
Feb. 16	First day post-respirator	9.1	11.1	Two minutes of inhalation
Feb. 21	Post-respirator		15.5	Second minute
			18.5	Third minute
			20.3	Fourth minute

Table VI gives the results of ventilation measurements and alveolar gas analyses made on January 25, 1951, almost one year after respirator

TABLE VI

Observations 11½ months after respirator treatment (N. G., January 25, 1951)

Gas inhaled	Minute volume BTPS	Respiratory rate	Tidal volume	Alveolar	
				pCO_2	pO_2
	liters per min.		*cc.*	*mm. Hg*	*mm. Hg*
Room air	11.6	20	580	34	113
99+% oxygen for 10 minutes	10.0	19	526	32	650 (approx.)
5% carbon dioxide in air 3rd and 4th minutes	12.8	19	674	50	—

Expired air: 2.55% CO_2, 18.38% O_2, R.Q. 1.
CO_2 dead space: 225 cc.

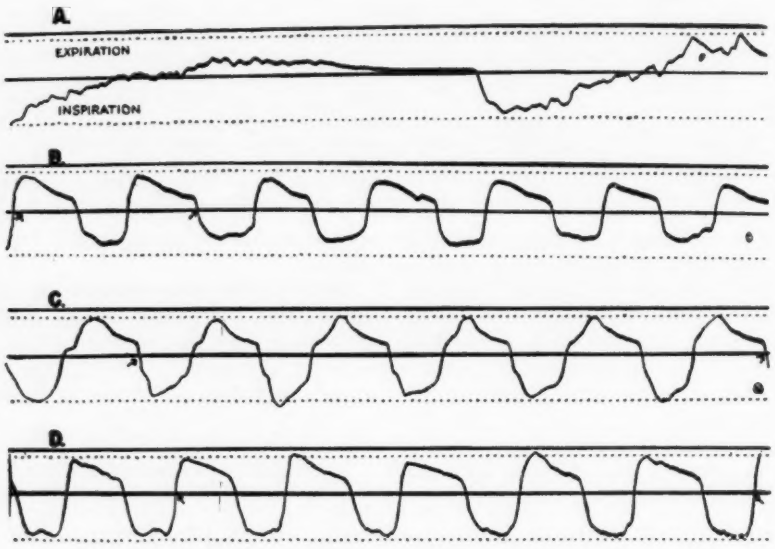

FIG. 1. INSTANTANEOUS AIR FLOW RATE RECORDINGS

A. Spontaneous breathing before respirator treatment. Rate very slow; successive expiratory volumes differ, indicating changing midcapacity. Many of superimposed irregularities of pattern are synchronous with heart beat.

B. Respirator cycling from + 5 cm. to − 4 cm. H_2O. Inspiratory pattern changes (below base line) indicate that the patient was not being passively breathed by the respirator. Sustained expiratory flow rate suggests an increased airway resistance.

C. Respirator cycling from + 10 to − 12 cm. H_2O. Pattern of air flow related to known pressure pattern of this type of respirator indicates that the patient is no longer combatting the respirator.

D. Spontaneous breathing pattern after respirator treatment. Record is very much like that of *B* above. Expiratory flow pattern again suggests increased airway resistance and large functional residual volume.

treatment. There was no significant difference between ventilation rates and alveolar pCO_2's when the patient changed from room air to oxygen breathing. Carbon dioxide administration produced some increment in ventilation, but this represents only the average for the third and fourth minutes, and the observation should have been made for a longer period. In a subsequent test on February 17, 1951, the ventilation increased from a resting value of 8.37 liters per minute to 14.41 liters per minute at the sixth minute of inhalation of 5.16 per cent carbon dioxide in air.

Figure 1 is a pneumotachogram from this patient. The expiratory pattern of air flow is consistent with the presence of emphysema and some degree of obstruction. The irregularities early in the expiratory phase suggest active muscular effort; the prolongation of expiration and sharp fall to base line at the end of expiration suggest the presence of increased airway resistance, which slows flow and increases the amount of air retained in the lungs.

Since leaving the hospital this man progressed satisfactorily. Following a period of rest at home he returned to his job in a candy factory where he has now worked continuously for more than nine months. He is free of the headaches which were formerly a major symptom, and is able to climb a flight of stairs with only slight dyspnea.

The second patient (J. M.) was found to have an alveolar pCO_2 of 66 mm. Hg on July 1, 1950. Examinations between July 8 and 12 showed ar-

terial pCO_2 values at 51 and 61 mm. Hg on two occasions, with an alveolar pCO_2 averaging 53 mm. Hg. At this time the administration of 90 + per cent oxygen consistently reduced ventilation by about 25 per cent within five minutes. The minute volume on room air was 6.5 to 7 liters. When high oxygen was given, the alveolar pCO_2 rose rapidly to an average of 75 mm. Hg, while the ventilation fell. The arterial pH at this time was 7.36, falling to 7.23 upon administration of oxygen. The arterial pO_2 was consistently 35 to 37 mm. Hg, giving an arterial oxygen saturation of about 65 per cent on room air. Residual air and maximum breathing capacity determinations were valueless because of poor co-operation of the patient. The best measurement of vital capacity was about 900 cc. When a mixture of 94.75 per cent oxygen and 5.25 per cent carbon dioxide was administered for five minutes, there was a progressive reduction in ventilation.

Respirator therapy was started on July 12, and continued for five days. A short preliminary treatment period of eight hours had been shown to confer no benefit.

Efforts to increase ventilation proved difficult, even when a pressure change of 30 cm. of water was produced in the respirator and various other devices were employed to encourage breathing. The patient appeared to resist the action of all these devices by skillful use of his glottis. Nevertheless, arterial and alveolar pCO_2 showed a downward trend. Three determinations of the alveolar pCO_2 averaged 41 mm. Hg for the period July 14 to 16. At the same time three determinations of the arterial pCO_2 averaged 40 mm. Hg. The alveolar pO_2 was 106 mm. Hg and the arterial pO_2 had risen to 55 mm. Hg (average of four determinations). This gave an arterial-alveolar difference of 51 mm. Hg.[2] The arterial oxygen saturation on room air was 85 per cent.

The patient was removed from the respirator on July 17, but did not maintain the above relatively satisfactory condition. In the course of the two following days the arterial pCO_2 rose to 50 and then to 64 mm. Hg while the pO_2 descended to 28 mm. Hg which gave a saturation of only 48 per cent on room air. The arterial-alveolar oxygen difference rose to 62 mm. Hg.

[2] Normal value in subject at rest, sitting, breathing room air at sea level: 5 to 14 mm. Hg.

It is of interest that on the first day out of the respirator the patient exhibited no diminution in breathing upon administration of 90 + per cent oxygen. Some 36 hours after leaving the respirator, the former response of diminution in breathing was again observed clinically, and this was confirmed the following day with more precise measurements.

The arterial pH, which was on the acid side of normal with the patient on room air before respirator treatment, became alkaline during residence in the respirator, but fell to normal following administration of chlorides to the patient. Coincident to the administration of chlorides, the patient appeared to breathe better with the machine.

DISCUSSION

Pathologic physiology

A decrease in ventilation during administration of oxygen appears to have been observed first by Mosso (18) who described such a depression in animals anesthetized with morphine and chloral; this work has since been extended by others (8, 19). Beddard and Pembrey (20) and Campbell, Hunt, and Poulton (21) described a similar ventilatory response in some patients with pulmonary and cardiac disease.

Barach and Richards (22) described this respiratory response to oxygen in a patient in whom the arterial pCO_2 rose from 48 to 67 mm. Hg during oxygen therapy. Similar results have been reported by Berconsky (23), Barach (24), Taquini and co-workers (25), and by Baldwin, Cournand, and Richards (5). Barach (2) has noted the ill effects of oxygen upon certain patients with emphysema and has recommended a gradual increase in percentage of inhaled oxygen. André (3) reviewed "apnea produced by oxygen" and concluded there was a case to be made for a multiple therapy including oxygen, carbon dioxide, analeptics, and artificial respiration provided the combination did not overtax the patient! Donald (6) has observed a patient with emphysema and heart failure who deteriorated into coma during oxygen therapy while the arterial pCO_2 rose rapidly to 120 mm. Hg. After withdrawal of oxygen the pCO_2 value fell rapidly and his general condition improved. Comroe, Bahnson, and Coates (7) reported eight patients with emphysema and chronic

hypoxia who deteriorated mentally when given oxygen. An increase of pCO_2 and a diminution in ventilation were accompanying features. All these patients initially had a pCO_2 of 50 mm. Hg or over and the phenomenon was seen in about half the patients who were found to have a pCO_2 in excess of 50. In two of these subjects, 10 per cent carbon dioxide was administered for sufficient time (five minutes) to raise the arterial pCO_2 higher than it had been raised by the diminution in ventilation in response to oxygen; nevertheless, they did not become sleepy. Comroe rightly suggested that further observations would be required to determine the role of carbon dioxide narcosis.

Davies and Mackinnon (26) observed that a rise in cerebrospinal fluid pressure accompanied the phenomenon of diminished ventilation and described a similar rise when carbon dioxide was inhaled. Papilledema has been described in some patients with advanced emphysema (27).

Thus it is well established that a small proportion of patients with advanced emphysema have carbon dioxide retention and that when the partial pressure of carbon dioxide exceeds 50 mm. Hg the administration of oxygen may affect the patient adversely. Deterioration is associated with a rising pCO_2 and elevation of cerebrospinal fluid pressure. Recognition of the syndrome depends upon evidence of advanced emphysema and in addition these special tests: a lowered arterial oxygen saturation, a high value for carbon dioxide tension in the arterial blood and alveolar air, a diminution in ventilation and rise in pCO_2 when oxygen is administered, and a poor ventilatory response to carbon dioxide.

It is not fully known how such emphysematous patients progress to the state of severe retention of carbon dioxide, but one likely factor is the element of airway obstruction present in many such cases. Some features of the pneumotachograph pattern of emphysema patients may be simulated by placing an obstruction in the airway of a normal subject; under these circumstances the work of breathing is plainly greatly increased.

Davies, Haldane, and Priestly (28) demonstrated the effect of anoxemia upon fatigue and failure of the respiratory center when there is resistance to breathing; they observed a rise in carbon dioxide tension during resistance breathing. This finding has been examined further by Cain and Otis (29) who remark that retention of carbon dioxide during resistance breathing indicates a compromise by the body; "the body tolerates some rise in carbon dioxide tension in preference to expending the effort that would be required to keep it at the original level." It is interesting to speculate whether pulmonary infections may contribute to the retention of carbon dioxide by diminishing muscular power, by increasing the work of breathing, or by reducing the sensitivity of the respiratory center.

Physiologic basis of therapy

The therapeutic objectives in cases of carbon dioxide retention are primarily three, to reduce the arterial pCO_2 to normal levels by promotion of adequate ventilation, to maintain this level of ventilation long enough for the patient to adjust to the altered level of carbon dioxide, and to encourage him to maintain this increased ventilation.

Although in many emphysematous patients it may not be difficult to increase the ventilation greatly, it is advisable to proceed cautiously and to approach gradually the level required to maintain a normal pCO_2. In this way there is sufficient time to permit adjustment of acid-base balance and to permit re-acclimatization of the respiratory center.

Acclimatization of the respiratory center has been observed at high altitude. Douglas and coworkers (30) showed that after ascent from sea level to Pike's Peak (barometric pressure 460 mm. Hg), the pCO_2 fell immediately from an average of 39.3 to an average of 33.6 mm. Hg, and the pO_2 from 104.7 to 48.4 mm. Hg. After six days at the summit, the alveolar pCO_2 fell to 29.1 mm. Hg, and the pO_2 rose to 53.5 mm. Hg. Rahn and Otis (31) made similar observations upon subjects acclimatizing at 9,500 feet; they further demonstrated, by means of breath-holding tests and carbon dioxide inhalation, an increased sensitivity of the center to carbon dioxide after acclimatization. In addition, the breathing of high oxygen mixtures caused no immediate change in the alveolar pCO_2 and consequently no decrease in ventilation. Houston and Riley (32) found that subjects acclimatized to 22,000 feet maintained for four days a raised ventilation and lowered pCO_2 when returned to sea level. Brown and associates (33) observed changes in ventilatory responses

to carbon dioxide after normal subjects had been overbreathed for 24 hours in a body respirator.

Adaptation of normal subjects to a high carbon dioxide environment has also been observed. Schäfer (34) recorded the ventilation, pCO_2, and pH in man during prolonged exposure to 3 per cent carbon dioxide. He noted a movement upwards of pCO_2 and a diminution in sensitivity of the respiratory center; the latter was assessed by plotting ventilation responses to inhaled carbon dioxide and alveolar ventilation against pCO_2. After an initial fall, the pH returned to normal levels. Otis (35) has investigated the impact on normal man of three days exposure to 3 per cent carbon dioxide. There was progressive inattention and listlessness and, following emergence, hypoventilation, a raised pCO_2, and a diminished response to carbon dioxide, all of which gradually returned to normal.

Gray (36) has analyzed acclimatization on the basis of his multiple factor theory of respiration (37). After considering and rejecting factors other than the three chemical agents, hydrogen ion concentration, pCO_2 and pO_2, he notes the tendency of the first and last of these three to return to normal and concludes that changes in sensitivity to pCO_2 are most likely since the pCO_2 remains at an abnormal level. He remarks that the respiratory mechanism adapts itself to a prolonged disturbance in the pCO_2 level by changes in sensitivity of such degree as to restore a normal response to the abnormal level of pCO_2.

There is no dispute that high partial pressures of carbon dioxide are narcotic. Carbon dioxide has been used as an anesthetic agent both in normal (38) and psychotic patients (39). Dripps and Comroe (40) described occasional stupor in normal men breathing 10.4 per cent carbon dioxide for three to four minutes. The narcotic level in those with an already raised resting pCO_2 has yet to be measured.

Theoretically there is a case for an attempt to assist in the excretion of retained carbon dioxide and to hold the patient at a normal arterial carbon dioxide tension until adaptation of the respiratory center to this tension has taken place. In both these patients it was possible to reduce the arterial and alveolar pCO_2 to normal by artificial respiration although the second patient (J. M.) co-operated poorly with the methods employed. In the case of N. G. the lowered pCO_2 was maintained by his own efforts. Inhalation of 5 per cent carbon dioxide 11½ months later produced increments in ventilation and there was no longer a diminution in ventilation when oxygen was administered. Arterial oxygen saturations observed in the follow-up period were 92 per cent, compared with the 82 per cent seen in 1947. In the case of J. M., the lowered pCO_2 level was not maintained by his own efforts. The first day after his period of artificial respiration there was no appreciable diminution in ventilation when oxygen was given; on the second day the pCO_2 had risen and the oxygen diminution response was again seen.

The reasons for success of respirator treatment in one patient and failure in the other are not wholly clear. In the patient who later died, the level of carbon dioxide concentration was not nearly so high, and therefore contributed less to the total picture; the degree of cardiac and renal failure was pronounced and probably accounted for the fatal termination. Both patients were almost moribund when placed in a respirator. The one who eventually recovered was convinced, during relatively rational periods, that he was going to die and that the respirator would be to no small extent responsible. Nevertheless, the therapy was not given up, and eventually the remarkable improvement took place. It appeared likely, but could not be proved, that the change in carbon dioxide levels accounted for the dramatic reversal of the disease picture. It was, furthermore, expected that regression would soon occur; such was not the case although the patient received no breathing exercises or any further mechanical assistance. There were no significant changes in physical signs in the chest, in chest roentgenograms, or in pulmonary volume subdivisions. The long-lasting symptomatic improvement has appeared to be simply the result of an increase in pulmonary ventilation sufficient to maintain nearly normal gas exchange.

In the case of N. G. other possible causes of, or contributions to, his dramatic recovery have been considered. Pulmonary infection could not be definitely identified or ruled out, but was regarded as not a significant factor since the patient received substantial daily penicillin therapy throughout his hospital stay, the body temperature and erythrocyte sedimentation rate before and after

respirator therapy showed no significant variation, and chest radiographs before and after respirator treatment showed no significant change. There was no evidence of pulmonary edema immediately prior to respirator treatment.

Amphetamine sulphate was administered daily between February 17 and March 29, 1950. This substance has been shown to be a mild respiratory and metabolic stimulant (41, 42), but termination of its use in this patient had no noticeable effect.

SUMMARY

1. A study of two patients with emphysema and carbon dioxide retention showed diminution of ventilation and elevation of pCO_2 upon administration of oxygen, and no increment in ventilation when a mixture of 5.25 per cent carbon dioxide in oxygen was breathed.

2. Increase in lung ventilation brought about by use of a body respirator reduced the pCO_2 to normal values in both subjects, while the arterial pO_2 and oxygen saturations were markedly raised above previous levels.

3. Subsequent to respirator treatment one patient has for 12 months maintained his pCO_2 at normal levels, and his responses to administration of high oxygen or carbon dioxide mixtures have been characteristic of the response of normal individuals. The other patient showed no diminution in ventilation while breathing oxygen in the immediate post-respirator period, but the improvement was very short in duration.

4. More impressive than the biochemical response in patient N. G. was the clinical improvement which immediately followed the respirator treatment. After more than three years of severe headaches and marked exertional dyspnea, he became free of headaches and noted respiratory distress much less frequently. He returned to work, and at the present time has been at his job continuously for over nine months.

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PULMONARY FUNCTION STUDIES IN SARCOIDOSIS[1]

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In spite of a considerable literature on sarcoid disease, very little has been written concerning alterations in pulmonary physiology produced by this condition. Baldwin, Cournand, and Richards (1) recently reported studies of pulmonary function in various fibroses of the lungs, including two proven and three presumed cases of sarcoid. However, no patient was included in their study if the residual volume of the lungs exceeded the predicted normal value.

We have had the opportunity of studying pulmonary function in a group of ten patients with sarcoidosis. The clinical manifestations of the disease included involvement of lymph nodes, lungs, liver, skin and bone. The diagnosis was confirmed in each case by biopsy of a lymph node or cutaneous lesion. All had negative tuberculin skin test. Eight had demonstrable involvement of the lungs or of mediastinal lymph nodes (see Table I). We are presenting the data obtained on these to illustrate a pattern of pulmonary impairment that may be found in this disease.

METHODS

Pulmonary function studies were performed on each patient when first seen and, in five patients, were repeated two months later. The following measurements were made:

1. Arterial blood was obtained with the patient at rest, breathing room air. Arterial pH was measured at 37° C. in a Cambridge closed glass electrode. The CO_2 content of whole blood was measured and CO_2 pressure was calculated (2). Arterial O_2 content and capacity were measured (2) and O_2 saturation was calculated after making appropriate corrections (3).

2. Intrapulmonary distribution of inspired O_2 was studied by the single breath technic described by Fowler (4) and Comroe and Fowler (5) using the Lilly nitrogen and flow meters (6, 7). Data were derived from analysis of curves obtained from a single maximal expiration following a single maximal inspiration of O_2. Studies of intrapulmonary gas mixing were also made by the technic of Cournand and his associates (8), *i.e.*, measurement of the N_2 concentration in an alveolar gas sample delivered at the end of a seven-minute period of O_2 inhalation.

3. Vital capacity, inspiratory capacity (maximum volume of gas that can be inspired from resting expiratory level, and expiratory reserve (maximum volume of gas that can be expired from the resting expiratory level) were measured separately on a Benedict-Roth spirometer; the maximum of three trials was recorded. Functional residual capacity (FRC—sum of RV and expiratory reserve) was determined by the open circuit method of Darling, Cournand, and Richards (9). Residual gas volume (RV) and total capacity (TC) were calculated from the above values.[2] Maximal breathing capacity per minute (MBC) was determined by maximal voluntary hyperventilation for 15 seconds through low resistance valves and wide tubing. The expired gas was collected in a compensating Tissot spirometer of 140 L capacity. The maximum of three attempts was recorded. Respiratory rate, depth, and minute volume were measured while the resting patient breathed through a mouthpiece and valves into the Tissot spirometer.

All gas volumes were corrected to 37° C. 760 mm. Hg, saturated with water vapor. Predicted values for maximal breathing capacity were based on the regression formula of Baldwin, Cournand, and Richards (11). The predicted values for lung volumes were derived from a composite summary of the literature, in which height and weight were the important variables.

RESULTS

The results of the pulmonary function tests are presented in Tables II–IV.

A. Patients with hilar and mediastinal adenopathy. In three patients (O. H., B. S., and I. W., Table I), radiologic studies demonstrated that the intrathoracic lesions were confined largely to enlargement of the hilar and mediastinal lymph nodes. In these, the physiological findings were essentially within the normal range except for a slightly reduced MBC in patient I. W. and low arterial O_2 saturation in patient O. H.

B. Patients with parenchymal involvement of the lungs. Five such patients were studied. Vital

[1] This study was supported by a grant from the Commonwealth Fund.

[2] The terminology used in this paper is that proposed by Pappenheimer (10).

TABLE I

Clinical data upon patients with sarcoidosis

Patient	Sex	Age	Ht.	Wt. (*lbs.*)	S.A.	Radiologic evidence of pulmonary disease	Symptoms related to respiratory disease
O.H.	M	17	5′7″	127	1.67	Mediastinal and hilar adenopathy, plus slight, diffuse reticulation in lung fields	None
B. S.	F	24	5′6″	127	1.63	Mediastinal and hilar adenopathy	None
I. W.	F	29	5′8″	176	1.93	Mediastinal and hilar adenopathy	None
S. F.	F	58	5′3″	170	1.84	Linear densities, chiefly in lower thirds, both lungs	Mild dyspnea on exertion
M. M.	F	44	5′5″	127	1.63	Flocculent and linear densities in upper and lower thirds right lung and upper third left lung	None
G. O.	F	33	5′3″	100	1.47	Extensive densities in upper third of each lung	None
E. S.*	F	26	5′1″	84	1.36	Flocculent and linear densities entire left and lower half right lung. Huge cyst in upper right lung enlarging over several years	Dyspnea on exertion
J. W.	F	28	5′2″	103	1.49	Widespread flocculent and linear densities distributed uniformly throughout both lungs	Dyspnea on exertion

* Died following an intrabronchial hemorrhage 10 months after studies were performed.

capacity was reduced significantly in three of the five; this was due largely to a diminution in the inspiratory capacity. Total lung capacity was less than the predicted normal value only in patient J. W. In all five, residual volume was increased markedly; it varied from 160% to 331% of the predicted normal value. The RV/TC ratios were all abnormally high, 30% to 65%. The FRC was also greater than normal in all. Thus the lungs were in a hyperinflated state at the end of both a forced and a normal expiration. All five had evidence of impairment in the mechanics of breathing; the MBC was 56% to 74% of expected normal values.

All had definite abnormality in the distribution of inspired gas to the alveoli as judged by the single breath analysis (5), even though pulmonary nitrogen elimination was normal in all five as judged by the Cournand seven-minute test (8). This emphasizes the greater accuracy and reliability of the single breath test as a measure of the uniformity of distribution of inspired gas. The reasons for this are presented elsewhere (5); hyperventilation during the seven-minute test was probably suffi-

TABLE II

Arterial blood measurements

Patient	O_2 cont.	O_2 cap.	O_2 satur.	CO_2 cont. (wh. bl.)	CO_2 cont. (plasma)	CO_2 pressure	pH	Total Hb	Hematocrit
	vol. %	*vol. %*	%	*vol. %*	*vol. %*	*mm. Hg*		*grams*	%
O. H.	17.2	18.2	92.3	50.1	59.8	40.5	7.41	13.6	46
B. S.	16.9	17.4	96.7	50.9	60.5	39.0	7.44	13.0	42
I. W.	14.5	15.1	96.2	54.0	62.4	42.2	7.41	11.3	35
S. F.	18.9	19.8	95.5	37.8	46.2	29.2	7.45	14.8	43
M. M.	13.7	14.3	96.1	47.2	53.9	40.0	7.38	10.7	35
G. O.	14.7	15.3	96.0	48.5	56.2	38.2	7.42	11.4	34
E. S.	13.0	14.0	93.5	41.9	47.9	30.0	7.45	10.5	36
J. W.	17.0	17.9	95.1	44.0	51.6	41.0	7.34	13.4	—

TABLE III

Lung volumes in patients with pulmonary sarcoidosis

Patient	Vital cap. (cc.)			Insp. cap. (cc.)			Exp. res. (cc.)			Resid. vol. (cc.)			Total cap. (cc.)			% RV/TC	FRC		
	Obs.	Pred.	%	Obs.	Pred.	%	Obs.	Pred.	%	Obs.	Pred.	%	Obs.	Pred.	%		Obs.	Pred.	%
*O. H.	4,020	4,270	94	2,715	3,435	79	1,595	835	191	915	1,070	86	4,935	5,340	93	19	2,510	1,905	132
*B. S.	3,165	3,340	95	2,230	2,505	89	1,025	835	122	1,080	835	129	4,245	4,175	102	25	2,105	1,670	125
*I. W.	3,000	3,460	88	2,050	2,615	79	1,010	845	120	1,000	870	115	4,000	4,330	92	25	2,010	1,715	117
S. F.	2,420	2,560	95	2,000	1,920	104	500	640	78	2,150	900	239	4,570	3,460	132	47	2,650	1,540	172
M. M.	2,350	3,320	71	1,720	2,490	69	760	830	92	2,310	1,020	226	4,660	4,340	107	50	3,070	1,850	166
G. O.	2,990	3,220	93	1,960	2,415	81	1,530	805	190	1,290	805	160	4,280	4,025	106	30	2,820	1,610	175
E. S.	1,420	3,160	45	1,020	2,370	43	685	790	87	2,650	800	331	4,068	3,960	103	65	3,335	1,590	209
J. W.	1,090	2,860	38	630	2,145	29	480	715	67	1,220	715	171	2,310	3,575	65	52	1,700	1,430	118

* Pulmonary disease limited primarily to mediastinal and hilar adenopathy.

cient to mask the presence of the poorly ventilated areas, clearly revealed by the single breath.

Only one patient, E. S., had pulmonary insufficiency at rest for the oxygenating mechanism; none had insufficiency so far as CO_2 elimination was concerned. None had disabling dyspnea. Apparently breathing could still be increased, without severe discomfort, sufficiently to arterialize the venous blood.

DISCUSSION

The data obtained in our studies differ strikingly from those reported by Baldwin, Cournand, and Richards (1). Their patients with sarcoidosis had a mean decrease in residual volume and total capacity, whereas ours had no consistent change in total capacity but a marked increase in residual volume. The discrepancy appears to lie entirely in the selection of cases. Baldwin was studying diffuse pulmonary fibrosis specifically, and consequently included in her report only cases that fitted her rigid criteria. On the other hand, we were studying patients who fitted rigid clinical criteria for pulmonary sarcoidosis. The pathological disturbance in all cases of sarcoidosis may well be that of fibrosis, but as Spain (12) has pointed out, fibrotic lesions may occur predominantly in one of five general regions in the lung: bronchiolar, interstitial, intra-alveolar, vascular or pleural. In the cases reported by Baldwin, intra-alveolar or interstitial fibrosis must have been the predominant lesion, leading to obliteration of some functional lung tissue and a decrease in lung volume. In our cases, the fibrosis must have been concentrated around bronchioles or air ducts, so as to produce residual hyperinflation quite similar to that reported by Beale, Fowler, and Comroe (13) in symptom-free patients with chronic

TABLE IV

Alveolar gas uniformity and ventilatory measurements

	Alveolar gas uniformity % N_2	N elim. (9) % N_2	Resp. rate per min.	Resp. depth L/M²	Min. vol. (air) L/M²	Min. vol. (O_2) L/M²	MBC L/M²		
							Obs.	Pred.	%
Normals	1.5 or less	<2.5	16	.22	3.5	3.8			
Patient									
O. H.	1.0	1.5	11	.431	4.73	5.09	98.8	77.6	127
B. S.	1.5	1.0	24	.202	4.90	5.46	62.2	60.3	103
I. W.	1.5	1.0	19	.208	3.99	4.09	38.6	57.5	67
Patient									
S. F.	4.5	1.3	11	.516	5.65	5.27	27.9	43.3	66
M. M.	4.0	1.3	15	.294	4.42	4.47	37.1	50.4	74
G. O.	8.0	1.8	23	.224	5.24	6.25	41.6	55.6	75
E. S.	7.0	1.3	33	.305	10.10	9.94	32.8	58.8	56
J. W.	6.0	1.2	33	.235	7.85	6.04	40.1	57.5	70

asthma. It is likely that the clinical picture of pulmonary sarcoidosis varies sufficiently in different patients and at different stages of the disease that either alveolar fibrosis or bronchiolar fibrosis might occur, each producing a different physiological pattern. In our group, diffuse alveolar fibrosis may have been present in only one patient (J. W.). This one patient was quite similar to the cases of fibrosis reported by Baldwin in that the vital capacity was reduced to a much greater extent than the MBC and the TC was reduced to 65%; however, the patient had an RV 171% of predicted normal, which suggests a mixed picture of alveolar and peribronchiolar fibrosis.

It is possible that the parenchymal disturbance in sarcoidosis may, in some patients and at some stage of the disease, lead to the tearing of alveolar septa and diminution in elasticity that is characteristic of emphysema. We cannot be certain, without histologic studies or measurements of intrapleural pressure, that our patients did not have this destructive type of emphysema. However, we have the impression that our patients differed in several respects from patients with severe enough emphysema to result in residual volumes in the range of 47–65% of TC. Our patients did not have history of chronic cough or asthmatic attacks, elevated alveolar N_2 concentration after seven minutes of inhalation of O_2, decrease in arterial O_2 saturation such as occurs when there is uneven distribution of both blood and air to alveoli,[3] or elevation of arterial pCO_2 such as usually occurs in patients with advanced emphysema. Therefore we believe that the lungs of our patients were hyperinflated primarily because of obstructive lesions rather than because of primary destruction of alveolar septa and diminution in pulmonary elasticity.

Considered alone, pulmonary function studies are not useful in diagnosis of pulmonary sarcoidosis because the pattern may vary widely. However, we have found these studies to be helpful in assessing the type and degree of existing functional abnormality, and in measuring changes in function, whether due to natural evolution of the disease or as a result of therapy. Roentgen examination in this particular group of patients has been unreliable as a guide to functional impairment; we have observed that considerable change may occur both in function studies and in clinical symptoms with little change in the X-ray appearance.

[3] An exception here was patient E. S.; autopsy later revealed that she had distortion and scarring of bronchioles but in addition had many cystic-like areas which had resulted from destruction of parenchymal tissue.

SUMMARY

1. Pulmonary function studies were performed in eight patients with clinical sarcoidosis, confirmed by biopsy.

2. In three of these, the thoracic disease was confined primarily to hilar and mediastinal adenopathy; in these the function studies were essentially normal except for a decrease in maximum breathing capacity in one and a reduction of arterial O_2 saturation in another.

3. In five, the intrathoracic lesions were primarily pulmonary in nature; consistent abnormalities found in these were: (*a*) abnormal intrapulmonary gas distribution as measured by the single breath test; (*b*) increase in the absolute and relative value for residual capacity (residual hyperinflation); (*c*) decrease in maximum breathing capacity.

4. Reasons are presented for attributing these changes to peribronchiolar fibrosis rather than to intra-alveolar or interstitial fibrosis or to emphysema of the destructive type.

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THE AMOUNT AND NATURE OF URINARY PROTEINS IN NORMAL HUMAN SUBJECTS [1, 2]

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Glomerular filtrate and normal urine have generally been regarded as being protein-free. The presence of protein in the urine has long been considered as indicative of renal disease. The classical experiments of Wearn and Richards (1), demonstrating the absence of protein in the glomerular filtrate and urine of the frog, added impetus to this belief.

A number of investigators have taken issue with the generally held concept that the urine is protein-free including Möerner (2) who found 22–78 mg. per liter, Gunton and Burton (3) who declared the mean concentration to be 3.7 mg. per cent and Addis (4) who states 10 mg. of protein per 24 hours is normally excreted. The nature and exact amount of protein in urine, however, continues to be obscure.

The application of two technics, *a*) concentration of the urinary proteins by ultrafiltration (5) and *b*) analysis of the electrophoretic migration patterns using the Tiselius apparatus, has permitted us to approach the problem of the nature and amount of the proteins contained in normal urine. Ultrafiltration of urine through a collodion membrane, as routinely applied in our laboratory to recover urinary gonadotrophins (6), permits the concentration of protein from a large volume of urine. Sufficient protein can be concentrated in this way from a 72 hour aliquot of urine to have enough to submit to electrophoretic analysis.

MATERIALS AND METHODS

Subjects consisted of 12 normal male and three normal female students 24 to 30 years old. The women collected urine during the first half of the menstrual cycle. Each subject collected the total urinary output for a 72 hour period. Preservatives were avoided. The urine was refrigerated immediately and kept in the refrigerator throughout the period of collection. Specimens were delivered at the end of each 24 hour collection period, immediately filtered through paper to eliminate formed elements, found to be free of gross protein by the sulfosalicylic acid test, and run through the ultrafilter. These procedures and all subsequent concentration procedures were conducted in the cold room at 0° C. to keep denaturation at a minimum.

In order to establish that the proteins retained on the collodion membrane after ultrafiltration could be quantitatively recovered without alteration, several methods of extraction were compared, using the same pooled urine. The proteins were directly eluted from the membrane at pH 7 with *a*) distilled water, *b*) 0.14 M saline, and *c*) 1.0 M saline, or *d*) eluted from the dry powder residue remaining after the collodion membrane had been dissolved and extracted with alcohol-ether. In the latter method the proteins were reprecipitated with 5 volumes of 95 per cent ethanol, before dissolving in veronal buffer at pH 8.6, ionic strength 0.1. In each method the protein solution was dialyzed against veronal buffer before being subjected to electrophoresis. Eluting the proteins directly from the membrane with distilled water gave a lowered recovery of γ_1, γ_2 and β globulins, otherwise each method was identical qualitatively and quantitatively. Since desoxyribonucleoproteins are insoluble in 0.14 M saline, the fact that identical results were obtained suggests that desoxyribonucleoproteins are not present.

The following procedure for recovering the protein collected on the membrane was therefore adopted: The membrane was placed in a 15 ml. centrifuge tube and dissolved with 15 ml. alcohol-ether (1 part absolute alcohol and 1 part anhydrous ether), centrifuged and the residue washed three times with alcohol-ether, once with anhydrous ether, dried and stored at 0° C. After the entire 72 hour collection had been subjected to this treatment, each of the three tubes, containing residue from 24 aliquots each, was extracted three times using 2 ml. of 1.0 M saline each time. The pooled supernatant fluid was placed in a cellophane bag and dialyzed against running water (4° C.) for 24 hours. For each 10 ml. of dialyzed supernatant, 2 ml. of saturated saline solution were added, and the proteins precipitated with 5 volumes of 95 per cent ethanol. The precipitate was recovered by centrifugation, washed with absolute alcohol and once with anhydrous ether and dried.

In order to compare the urinary proteins with those of

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[2] Presented in part before the Northwest Group of the American Federation for Clinical Research, Vancouver, British Columbia, April 21, 1951.

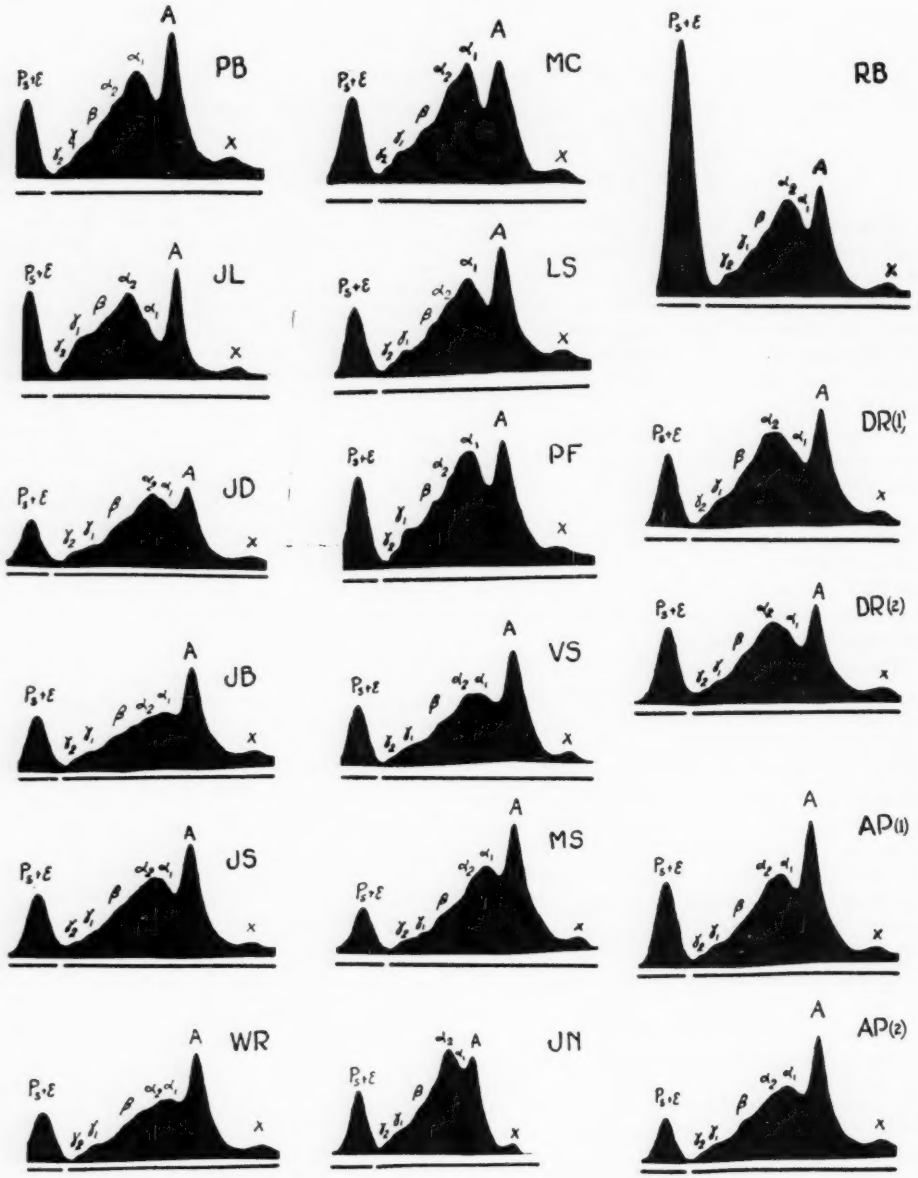


FIG. 1. ELECTROPHORETIC MIGRATION PATTERNS OF URINARY PROTEINS FROM NORMAL SUBJECTS

Taken after 120 minutes migration in veronal buffer, pH 8.6, ionic strength 0.1, potential gradient 7.4 volts/cm. The descending limb is depicted. The pattern on patient J. N. was taken at 90 minutes.

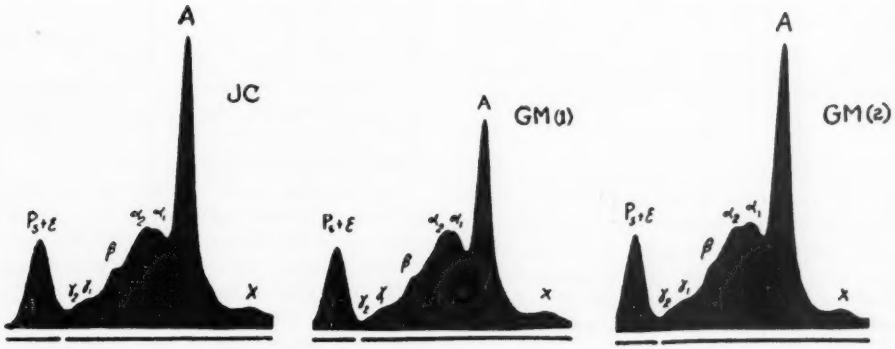


FIG. 2. ELECTROPHORETIC MIGRATION PATTERNS OF URINARY PROTEINS OF TWO PATIENTS HAVING ORTHOSTATIC ALBUMINURIA

One hundred twenty minutes of migration in veronal buffer, pH 8.6, ionic strength 0.1, potential gradient 7.4 volts/cm.

the blood serum, veronal buffer at pH 8.6, ionic strength 0.1, was used. This had been found to give the most satisfactory resolution of the blood serum proteins (7). The dry material was dissolved in 10 ml. of this buffer and dialyzed in a cellophane tube for 24 hours against 2 liters of the same buffer, changing buffer once during dialysis. Volume changed very little during dialysis so that the solution could be used directly in our analytical cell which requires 9.8 ml. (the cell with the long middle section). The electrophoretic analyses were performed with the Klett equipment using the Longsworth's scanning method for recording the patterns. The proteins were allowed to migrate for 120 minutes under the influence of a potential gradient of 7.4 volts/cm., at a temperature 0.5° C. Due to urinary pigments absorbed or held by stronger combination on the protein, infra-red

TABLE I

Four chemical tests for identification of urinary proteins in the different fractions

TEST / FRACTION	Biuret test.	Sulfosalicylic acid test.		Identification of albumins plus globulins by the acetic acid heat coagulation test.			Separation and identification of albumins from globulins by the acetic acid plus ammonium sulfate heat coagulation test.					
		Cold	Boiling	Addition of a slight excess of acetic acid.			Addition of acetic acid in slight excess.					
					Addition of 0.5 ml of satur. sol. NaCl per ml.			Add. of ammonia to alkaline reaction.				
					Cold	Boiling			Add. of ammonium sulfate to half saturation.			
									Precipitate		Supernatant	
										Extract with ac. acid and boil.	Add. acetic acid slight excess.	Supern. Boiling
Bottom	6+	5+	4+	1+	1+	5+	1+	-	4+	4+	1+	4+
I_A	2+	2+	2+	2+	-	1+	2+	-	-	-	-	1+
II_A	4+	4+	3+	1+	1+	4+	1+	-	2+	2+	1+	3+
III_A	5+	5+	4+	1+	1+	5+	1+	-	3+	3+	1+	3+
IV_D	-											
V_D	2+	2+	1+	-	1+	3+	-	-	2+	2+	-	-
VI_D	4+	3+	2+	-	1+	4+	-	-	3+	3+	-	±

The number in front of + indicates the color density or the amount of precipitate formed after completion of all steps indicated in top of each column. Negative results are indicated as—.

light and spectroscopic plates sensitized for the infra-red were used. Because of the low protein concentration the boundaries were recorded with a magnification factor $K' = 1/6$ (*i.e.*, the ratio of the speed of the photographic plate to that of the schlieren diaphragm was twice the usual).

RESULTS

Sufficient protein was concentrated from the 72 hour urine collection of each of the 15 normal control subjects to detect several protein components in the electrophoretic analysis. The most striking result was the uniformity of the pattern elicited. Note that not only were the patterns of males similar to each other but that females were in no way different.

It is also noteworthy that, whereas minor variations exist between patterns of one individual as compared to another, the pattern of a given individual tends to remain constant over several months time. This is illustrated by normal controls DR (1) and (2) and AP (1) and (2) in Figure 1 and by a subject with orthostatic proteinuria, GM (1) and (2) in Figure 2.

It was possible to calculate the total urinary protein output from the electrophoretic analyses. A mean value of 39.0 mg./24 hours was found. The extremes were 30.5 mg. and 49.6 mg.

It was further noted that whereas the relative amounts of protein excreted by the same individual remained constant, as judged by the electrophoretic patterns, the total output varied considerably from time to time. From Table II it can be seen that DR, whose pattern remained constant, excreted 35.5 mg. on one occasion and 43.9 mg. on another occasion some months later.

Analysis of the electrophoretic patterns

False boundaries represent buffer ion concentration differences existing between the protein and

TABLE II

Amount of protein, albumin and globulin contained in urine of normal male and female subjects and in orthostatic albuminuria

Subject	Sex	Total Protein mg./24hrs	Albumin mg./24hrs	Globulins mg./24hrs	A/G Ratio
PF	Male	46.3	14.1	32.2	0.44
LS	"	41.8	14.1	27.7	0.51
PB	"	43.7	16.1	27.6	0.58
JL	"	38.2	10.6	27.6	0.38
MC	"	49.6	17.1	32.5	0.52
JB	"	31.2	11.8	19.4	0.60
JS	"	38.1	13.5	24.6	0.55
WR	"	31.0	11.2	19.8	0.56
JD	"	32.8	10.6	22.2	0.48
RB	"	39.4	12.6	26.8	0.47
DR (1)	"	35.5	12.1	23.4	0.51
DR (2)	"	43.9	13.1	30.8	0.43
AP (1)	"	44.0	15.3	28.7	0.53
AP (2)	"	42.1	15.7	26.4	0.59
VS	Female	38.3	14.6	23.7	0.61
MS	"	41.2	14.6	26.6	0.55
JN	"	30.5	9.0	21.5	0.42
Average		39.0	14.8	25.8	0.51
Standard Deviation		5.7	2.2	3.9	
Cases having "orthostatic albuminuria"					
JC	Male	52.7	28.8	23.9	1.20
GM (1)	"	49.4	21.8	27.6	0.79
GM (2)	"	51.0	29.4	21.6	1.36

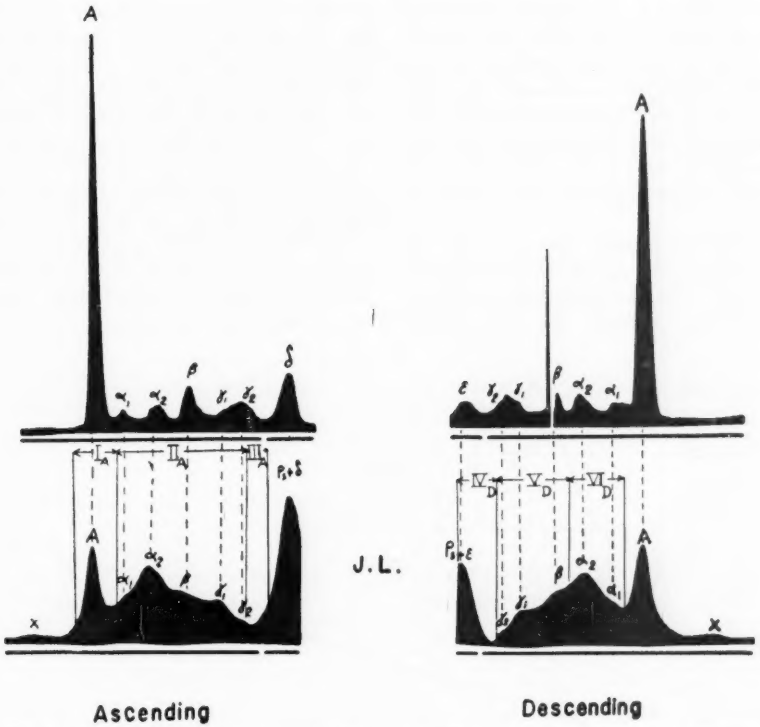


FIG. 3. COMPARISON OF ELECTROPHORETIC PATTERNS OF NORMAL HUMAN SERUM AND URINARY PROTEINS

Taken after 150 minutes migration in veronal buffer, pH 8.6, ionic strength 0.1, potential gradient 7.4 volts/cm. Patterns for serum are above, urine below.

buffer solutions. These are conventionally designated as ϵ for the descending limb and δ for the ascending limb. A larger false boundary than occurs in serum was noted in the patterns from the urinary concentrates. This was found to be due to a high molecular weight polysaccharide and therefore the false boundaries are designated as Ps + ϵ and Ps + δ, respectively.

The initial position of the boundaries is designated in the patterns by a break in the horizontal black line below each pattern.

Comparisons of urinary with serum patterns reveals the same components in each. This can be seen in both the ascending and descending limbs in Figure 3 after both serum protein and urinary proteins have migrated for 150 minutes under similar experimental conditions. The serum pattern was recorded at conventional speed of the photographic plates and the urinary pattern at double speed. The heights of the urinary peaks are consequently doubled.

It is striking that for each *serum* protein component represented in the pattern as a peak, a corresponding urinary component can be identified as having migrated the same distance in the same length of time under identical circumstances. Corresponding components have been designated by the identical label, *i.e.*, α_1, α_2, β, γ_1, γ_2 globulins and A, albumin (Figure 3), and to facilitate comparisons dotted lines have been drawn between corresponding components.

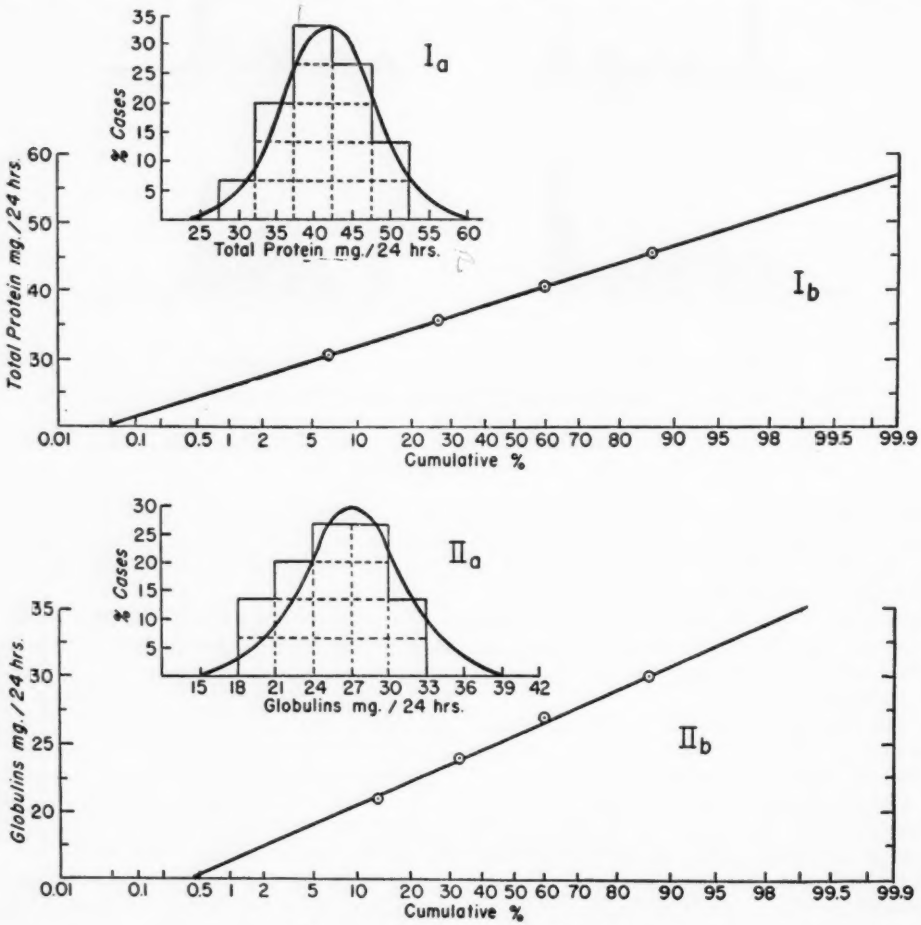
It is concluded that proteins having the same ranges of mobilities exist in normal human blood serum and in normal human urine.

Although qualitatively each serum protein appears to be represented in the urine, by a protein of similar mobility, the relative amounts of each protein in the urine varies considerably from that in serum. It is at once apparent that urine contains relatively more globulin than serum and relatively less albumin than serum. Thus the A/G ratio for urine is the reverse of that existing in serum.

The globulin components vary as well. In normal serum the β and γ_2 globulins predominate, while in normal urine the components labelled α_1 and α_2 globulins predominate. The urinary globulins are less homogeneous than the serum globulins. The heterogeneity may have been acquired during the ultrafiltration process or could be due to the coexistence of proteins with intermediate electrophoretic mobilities, which are either absent from serum, or present in very small amounts.

A component not found in serum but uniformly found in urine migrating more rapidly than albumin (A) is designated as X in the patterns.

The chemical behavior of urinary proteins separated by migration in the Tiselius apparatus. It would be a remarkable coincidence if totally different urinary proteins or even non-proteins mi-



(FIG. 4.—*Continued on next page*)

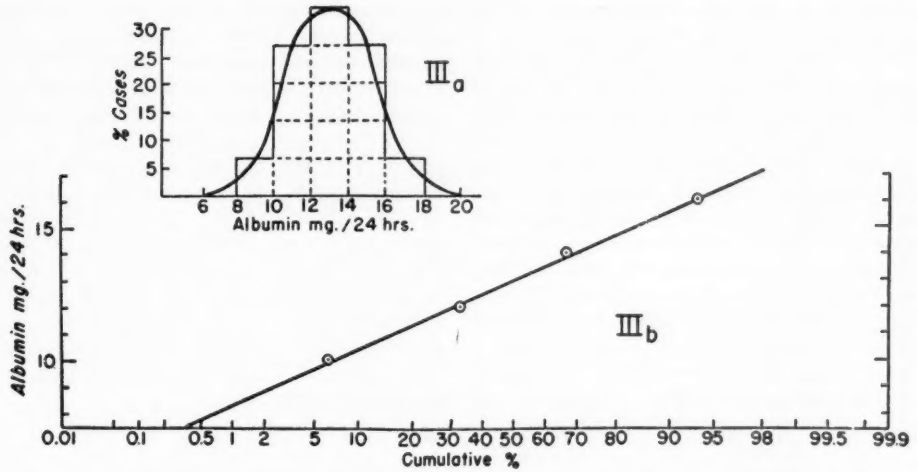


FIG. 4.—*Continued.* STATISTICAL DISTRIBUTION CURVES AND PROBABILITY LINES SHOWING GAUSSIAN DISTRIBUTION OF THE AMOUNT OF TOTAL PROTEIN, ALBUMIN AND GLOBULIN IN URINE OF NORMAL SUBJECTS

grated at the same speed as each of the serum proteins. Nonetheless, to exclude this possibility various fractions of the proteins were withdrawn from the electrophoresis cell at the end of 150 minutes of migration. This was possible after devising an instrument capable of accurately withdrawing a fraction of protein from the electrophoretic cell (8). This consisted of a motor-driven syringe attached to a needle long enough to reach any migrating component in the cell. Liquid was withdrawn slowly enough to prevent turbulence.

The fractions thus obtained are indicated in Figure 3 by the solid black vertical lines and designated as fractions I_A, II_A, and III_A, for the ascending limb and fractions IV_D, V_D, and VI_D, for the descending limb. Thus each fraction encompassed the following components:

I_A—Albumin A plus component X.

II_A—Albumin A plus α_1, α_2, β, γ_1, and some γ_2 globulin.

III_A—Mixture of all protein components plus X but excluding false boundary $P_s + \delta$.

IV_D—false boundary $P_s + \epsilon$.

V_D—false boundary plus γ_2, γ_1, β and some α_2 globulin.

VI_D—mixture of all components including false boundary but excluding albumin A and component X.

"Bottom fraction"—mixture containing all original components taken from bottom cell.

Each fraction was dialyzed in running water until free from buffer salts and then subjected to the various tests for proteins indicated in Table I, with the exception of fraction IV_D which proved to be devoid of protein by the Biuret test.

All other fractions proved to contain protein. Fraction I_A was positive for all tests in which albumin elicits a precipitate and negative to tests in which globulin alone elicits a precipitate. In addition the test for mucoprotein was positive. Since fraction I_A contained not only component A (albumin) but also component X, the reaction for mucoprotein may have been due to the presence of component X.

The remaining fractions were presumed to contain globulin and tested positively in each test in which globulins elicit a precipitate.

Fractions V_D and VI_D, presumed to contain globulin but not albumin, failed to react to a test in which albumin alone elicits a precipitate. These two fractions were devoid of reaction to tests which elicit a positive reaction for acid precipitable mucoproteins, but the sulfosalicylic acid test revealed that glycoproteins and acid soluble mucoproteins were also present. Since they contained neither component A nor X, it may be presumed that acid

precipitable mucoproteins were contained in one or the other of the latter fractions only.

None of the fractions contained desoxyribonucleoproteins since they were soluble in 0.14 M NaCl, *vide supra*. From phosphorus analysis it seemed unlikely that any nucleoproteins were present since it was calculated that approximately one phosphorus atom per protein molecule was present and if nucleoproteins were present to any extent more phosphorus would of necessity be detected.

Fraction IV_D (containing the false boundary only) while not a protein, proved to contain a nondialyzable substance which could be precipitated with 5 volumes of 95 per cent ethanol. It gave a dark brown color when mixed with concentrated sulfuric acid, and a positive Molisch reaction, which characterized it as a polysaccharide.

Quantitative analysis. Since the urinary concentrates contain polysaccharides in addition to the protein, dry weight will not yield information as to the total amount of protein in the urine. Such information was yielded by measuring the area of the electrophoretic pattern produced by protein components as a whole, and albumin and globulin separately, then applying the formula:

$$\text{Concentration} = \frac{K'}{K \cdot a \cdot b} \cdot A,$$

where $K' = 1/6$, a and b are constants of the apparatus and A is the measured area. K is the refractive index increment taken as $K = 0.001858$ (9) which is the value for human serum albumin corrected for the wave length $\lambda = 7200$Å of the light used, according to the formula:

$$K_\lambda = K_{5780Å}(0.940 + 2.00 \times 10^6/\lambda^2).$$

The values for total protein, albumin, globulin and A/G ratio are recorded in Table II. When subjected to statistical analysis it was found that each follows a normal distribution curve as seen from Figure 4. The total protein excreted per 24 hours thus has a mean value of 39.0 mg. and a standard deviation (estimated from the formula $\sigma = \sqrt{\frac{\Sigma\Delta^2 \cdot N}{N-1}}$) of 5.7 mg. For globulins the mean is 25.8 mg./24 hours, and standard deviation 3.9 mg. For albumin the mean is 14.8 mg./24 hours, and standard deviation 2.2 mg. The average A/G ratio is 0.51 as contrasted to the A/G ratio for normal serum of 1.15.

Two individuals (J. C. and G. M.) who were initially included with the normal control series exhibited patterns inconsistent with the remainder of the series. Albumin excretion was distinctly higher (falling out of the normal range, according to the Chavenet probability criterion), total proteins were elevated, but the proportion of the different globulin components were not at variance with the normal subjects (Figure 2). On closer questioning it was revealed that the clinical diagnosis of orthostatic albuminuria had been made on G. M. and although no such clinical diagnosis had been made on J. C., two siblings had the clinical diagnosis of orthostatic albuminuria. These cases are excluded from the distribution charts and listed separately in Table II.

DISCUSSION

The source of the urinary protein remains unclear. Experimental evidence exists to suggest that the renal tubules do not excrete protein but in fact tend to absorb protein when present. After producing renal damage Bieter (10) found that glomerular fish developed proteinuria whereas aglomerular fish did not. The reabsorption of proteins by tubules was demonstrated by Gerard (11) upon introducing several colloidal substances of different molecular weight into the proximal end of the proximal convoluted tubule of the salamander. He noted that the greater the molecular weight of the substance injected, the more distally it was absorbed. Dock (12), Gilson (13), Smetana (14, 15), and Oliver (16) concluded that protein is excreted by the glomerulus and absorbed by the tubules after injecting Evans blue dye (which combines with albumin) and other protein-bound dyes into rats.

Since proteins dyed with foreign substances might alter the permeability of the glomerular membrane, Addis (17) gave injections of rat serum to rats of the same strain and was able to produce proteinuria without producing renal damage. He ascribed the proteinuria to the raised protein concentration of the plasma. Similar observations have been made on dogs receiving injections of dog plasma (18) and in patients with liver cirrhosis after repeated intravenous injections of human serum albumin (19). This indirect evidence suggests that the proteins we detected in

urine are the result of glomerular filtration. The greater proportion of globulin in the urine may be due to greater tubular reabsorption of albumin.

SUMMARY

The quantity and nature of proteins in the urine of normal adults have been determined by applying the ultrafiltration concentration technic and electrophoretic migration analysis.

Components having the same electrophoretic mobility as those in normal serum were discovered to exist in normal urine, namely albumin, α_1, α_2, β, γ_1, and γ_2 globulin. The relative amounts of each differ for urine and sera in that the A/G ratio is reversed for urine. Whereas β and γ_2 globulins predominate in sera, the predominant globulins migrate with the same mobility as α_1 and α_2 globulins.

The mean normal excretion of protein is 39.0 mg./24 hours, for albumin 14.8 mg./24 hours and for globulin 25.8 mg./24 hours. The average A/G ratio is 0.51.

Mucoproteins and glycoproteins, as well as a polysaccharide and an unidentified component X, were found to be present in normal urine.

A technic is described for withdrawing various fractions of proteins from the electrophoretic cell after migration has occurred. Chemical analysis of the fractions confirmed that both albumins and globulins are excreted.

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"WATER DIURESIS" PRODUCED DURING RECUMBENCY BY THE INTRAVENOUS INFUSION OF ISOTONIC SALINE SOLUTION [1]

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Control of renal excretion of water in man is largely vested in the supra-optico-hypophyseal system. When the tonicity of the blood plasma and extracellular fluid rises, an increased secretion of antidiuretic hormone (ADH) leads to the conservation of water inasmuch as urinary solutes are excreted with a minimum of water. Conversely, when tonicity falls, a diminution of ADH output permits the excretion of a large volume of dilute urine. In both instances homeostasis is served.

Many stimuli, in addition to hypertonicity, increase ADH production, including certain anaesthetic (1) and narcotic drugs (2), barbiturates (3), smoking (4, 5) and nicotine (6, 7), acetylcholine (8), exercise (9), emotion (9), syncope (10), pain (11) and conditioned reflexes (11), as well as the direct electrical (12) or acetylcholine (13) stimulation of nerve cells and fibers connected with the pars nervosa of the pituitary gland. Removal of amounts of blood insufficient to alter blood pressure significantly may nevertheless lead to increased ADH activity (9). There is also suggestive evidence that there may at times be increased ADH activity in patients with cirrhosis of the liver (14, 15).

In contrast to the many factors which may lead to increased antidiuretic activity few other than destructive lesions involving the supra-optico-hypophyseal system and hypotonicity of the plasma and extracellular fluid have been reported to cause a decline in ADH activity. These are hypnotic suggestion (1), alcohol administration (16–18), and exposure to cold (19).

It is the purpose of this communication to present evidence that a rather rapid isotonic expansion of extracellular fluid volume (or some factor associated with such a volume increase) leads to a water diuresis of the type which follows diminished ADH activity.

METHODS

The subjects were three essentially healthy male adults hospitalized for the investigation of minor symptoms for which no organic cause was found and which cleared rapidly. Without previous preparation [2] they came to the laboratory one to two hours after breakfast, voided, and began the timed collection of urine specimens. When it became apparent that the flow of urine was diminishing, blood was collected without stasis from an antecubital vein after the arm had been immersed in hot water for five minutes to arterialize the blood (20), following which 3,000 ml. of 0.9 per cent solution of sodium chloride in water (Sp. Gr. 1.005 at 15° C.) was infused intravenously at a constant rate of 25 ml./min., the entire infusion requiring two hours. A blood sample was collected from the opposite arm immediately upon the completion of the infusion, and a third at a subsequent time. Urine was voided at intervals during and after the infusion, the subject standing for this purpose.

The volume of packed red blood cells was measured in Wintrobe tubes (21) and hemoglobin photometrically (22) on blood rendered incoagulable by a mixture of ammonium and potassium oxalate. Serum was obtained by centrifugalization of blood collected under oil. Serum total proteins were determined by gradient tube (23); chloride by the method of Sendroy as modified by Van Slyke and Hiller (24); creatinine by the Hare adaptation of Borsook's method (25); and sodium and potassuim by means of the Perkin-Elmer or Barclay internal standard flame photometer. Urine specific gravity was determined with a Westphal balance; chloride by the Volhard-Harvey method (26); sodium and potassium by the same

[1] Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

[2] The unforunate lack of a truly basal or steady state for the study of water and electrolyte excretion in man has been previously discussed (17). Subjects who have had no fluids after retiring on the preceding night are for the most part somewhat hydropenic on awakening in the morning and hence have enhanced antidiuretic activity.

method as employed for serum; and creatinine by Peter's modification of the Folin procedure (27). Urea and ammonia were determined together by the aeration method of Van Slyke and Cullen (28). The percentage change in plasma volume, $PV_2/PV_1 \times 100$, was calculated from the hemoglobin and hematocrit by the formula:

$$\frac{Hgb_1}{Hgb_2} \times \frac{1 - Hct_2}{1 - Hct_1} \times 100$$

and also from the serum total protein by the formula:

$$\frac{TP_1}{TP_2} \times 100.$$

RESULTS

Renal excretory rates for water and sodium, urinary specific gravity and solute concentrations, together with creatinine U/P ratio and clearance, are shown in Table I. During diuresis urine specimens were usually obtained for analysis every half hour but the results were averaged for intervals of one to two hours except when changes of significance occurred between the shorter periods. Determinations made on venous blood and calculated changes in plasma volume are given in Table II.

TABLE I

The effect of the intravenous infusion of isotonic saline solution on urine flow, concentration of solutes, creatinine U/P ratio, creatinine clearance, and sodium excretion rate

Subject date	Position	Time	Urine flow	Spec. grav.* (at 15° C.)	Na	Cl	K	Urea+NH_3 urea†	Creatinine		Na excretion
									U/P ratio	Clearance	
		min.	*ml./min.*		*meq./l.*	*meq./l.*	*meq./l.*	*mM/l.*		*ml./min.*	*micro-eq./min.*
E. F.	Recumbent	− 60 to 0‡	2.25	08	163	168	39	156	58	131	367
1/12/51	Recumbent	0 to 60(*1*)	2.08	10	164	183	33	170	67	139	341
	Recumbent	*61 to 120*	*7.08*	*02*	*86*	*88*	*5*	*33*	*13*	*92*	*609*
	Recumbent	121 to 150	9.08	05	119	120	4	31	12	109	1081
	Recumbent	151 to 240	3.55	08	196	200	18	82	34	121	696
1/9/51	Sitting	− 60 to 0	0.75	20	174	218	79	470	191	143	131
	Sitting	0 to 138(*2*)	1.70	12	187	238	72	220	82	139	318
	Sitting	139 to 180	1.67	09	176	242	60	219	82	137	294
	Recumbent	181 to 210	3.13	05	138	147	28	118	48	150	432
	Recumbent	*211 to 240*	*5.00*	*05*	*90*	*105*	*15*	*73*	*30*	*150*	*450*
S. C.	Recumbent	− 60 to 0	2.36	12	93	128	65	204	63	149	219
2/6/51	Recumbent	0 to 120(*3*)	3.39	06	82	96	33	114	40	136	278
	Recumbent	*121 to 180*	*8.34*	*02*	*64*	*66*	*12*	*47*	*18*	*150*	*534*
	Recumbent	181 to 270	4.11	09	173	183	29	103	39	160	711
2/9/51	Sitting	− 60 to 0	1.33	20	84	175	123	403	153	203	112
	Sitting	0 to 145(*4*)	1.38	15	126	194	92	273	110	152	174
	Sitting	146 to 225	3.63	12	144	189	57	157	66	240	523
	Recumbent	226 to 285	2.30	14	160	197	59	173	73	168	368
	Recumbent	*286 to 345*	*4.94*	*04*	*91*	*101*	*30*	*80*	*30*	*148*	*450*
E. B.	Recumbent	−105 to 0	1.24	16	130	146	42	260	101	125	161
1/24/51	Recumbent	0 to 119(*5*)	1.65	14	176	202	58	164	67	111	290
	Recumbent	120 to 180	3.00	13	217	238	38	114	42	126	651
	Recumbent	181 to 240	4.35	08	149	161	27	84	30	131	648
	Recumbent	*241 to 300*	*5.17*	*03*	*87*	*101*	*17*	*62*	*24*	*124*	*450*
	Recumbent	301 to 330	3.67	06	127	130	23	85	33	121	466
1/29/51	Sitting	− 97 to 0	0.93	20	198	241	77	318	146	136	185
	Sitting	0 to 119(*6*)	1.30	19	192	256	88	209	100	130	250
	Sitting	120 to 197	2.75	12	209	243	56	120	43	118	575
	Sitting	198 to 285	2.75	13	174	198	53	140	42	116	479
	Recumbent	286 to 315	3.66	07	116	132	31	104	36	132	425
	Recumbent	*316 to 347*	*6.56*	*03*	*75*	*77*	*20*	*35*	*17*	*112*	*492*
	Recumbent	348 to 375	5.00	04	109	113	27	58	25	125	545
	Recumbent	376 to 405	2.67	13	189	198	41	98	40	107	505

* The second and third digits after the decimal are shown.

† Urea and ammonia nitrogen were determined together but are recorded as if all the nitrogen was in the form of urea.

‡ Infusion begun at, and completed at (*1*) + 10, + 130, (*2*) + 15, + 135, (*3*) + 10, + 135, (*4*) + 19, + 135, (*5*) + 9, + 135, (*6*) + 17, + 130 minutes.

TABLE II

The effect of the intravenous infusion of isotonic saline solution on constituents of the blood and on the volume of the plasma

Subject Date Position	Time	Hematocrit	Hemoglobin	Total serum protein	Serum creatinine	Concentration in extracellular H_2O			$PV_1/PV_1 \times 100$	
						Na	Cl	K	(from Hct. and Hgb.)	(from T.P.)
	min.	*vol. %*	*gm. %*	*gm. %*	*mg./100 ml.*	*meq./l.*	*meq./l.*	*meq./l.*	%	%
E. F.	10	48.0	15.2	7.1	0.95	138.4	118.0	4.9	100.0	100.0
1/12/51	135	43.6	14.1	6.1	0.85	145.2	125.7	4.6	116.9	116.4
Recumbent	245	44.6	14.4	6.4	0.88	140.7	124.1	4.8	112.5	110.9
1/9/51	15	47.9	15.2	7.2	0.92	144.3	118.4	5.1	100.0	100.0
Sitting and recumbent	145	43.0	13.4	6.1	0.87	144.8	124.0	5.0	124.3	118.0
S. C.	10	47.0	15.2	6.5	1.06	141.5	118.8	4.4	100.0	100.0
2/6/51	133	43.0	14.0	5.7	0.94	145.0	125.9	4.4	116.8	114.0
Recumbent	223	41.0	13.2	5.7	1.06	143.7	123.2	4.3	128.2	114.0
2/9/51	17	49.1	15.8	7.2	0.91	138.9	116.3	4.7	100.0	100.0
Sitting and recumbent	145	42.0	13.7	5.8	0.87	141.7	121.4	4.9	131.4	124.1
	345	44.0	14.7	6.0	0.94	138.5	121.3	4.1	118.3	120.0
E. B.	9	41.7	13.1	7.0	1.28	147.9	118.9	4.8	100.0	100.0
1/24/51	143	37.3	11.6	6.1	1.13	145.4	124.6	4.4	121.5	114.8
Recumbent	338	38.2	11.9	6.3	1.10	143.8	125.2	4.7	116.7	111.1
1/29/51	15	42.3	12.8	7.0	1.26	144.4	120.1	5.4	100.0	100.0
Sitting and recumbent	135	36.9	11.3	5.9	1.12	146.1	124.8	4.6	123.7	118.6
	350	39.0	12.0	6.3	1.08	143.7	124.0	5.3	112.8	111.1

Infusion was begun immediately after the first blood sample was obtained and concluded just prior to withdrawing the second sample.

E. F. weighed 82 kg., S. C., 91 kg., E. B., 75 kg.

The rate of urine flow together with the value approximating the total electrolyte concentration, $2(Na + K)$, have been charted in Figures 1–3. In each experiment, during or following the infusion the rate of urine formation rose sharply and the concentration of electrolyte fell below that of extracellular water as well as below that of the infusion fluid. Specific gravity of the urine as well as the concentration of urea plus ammonia fell concomitantly with the decline in electrolyte concentration. The rate of sodium and chloride excretion rose in each instance during the saline infusion and

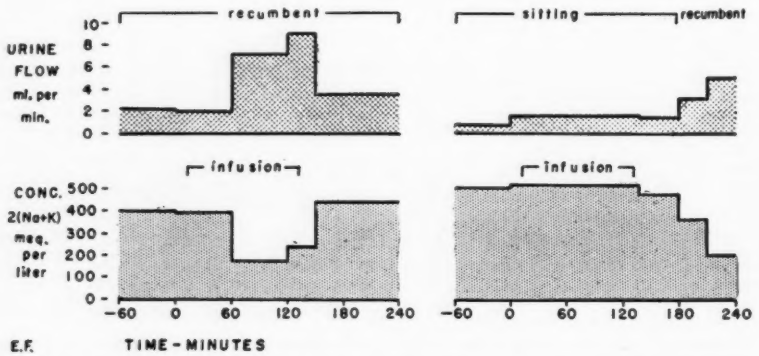

FIG. 1. ELECTROLYTE CONCENTRATION AND FLOW OF URINE IN RELATION TO THE INTRAVENOUS INFUSION OF ISOTONIC SALINE AND TO CHANGE IN POSTURE—PATIENT E. F.

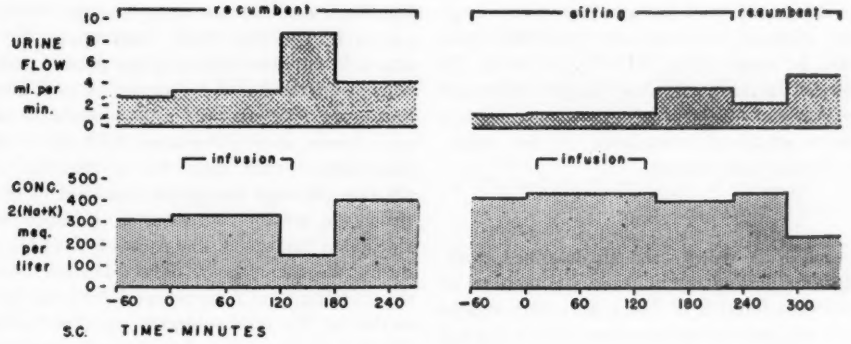

FIG. 2. PATIENT S. C.

Legend as in Figure 1.

continued well above the fore-period level throughout the entire post-infusion period. At the time that the urine was most hypotonic, the excretion rate of sodium and chloride averaged 2.6 times as great as in the fore-period.

It will be noted that in each instance with the subject sitting the urine flow was less and the electrolyte concentration greater during the fore-period than during recumbency. The creatinine U/P ratios were correspondingly higher with the subjects sitting. In the case of E. F., whose creatinine U/P ratio in the fore-period was 58 while recumbent, the most hypotonic urine was secreted during the second hour of the infusion and in S. C., tonicity was lowest during the first hour after the infusion. In the third subject, E. B., whose creatinine U/P ratio was considerably higher during the fore-period, and who was disturbed by the intravenous infusion (becoming pale and faint when the needle was inserted) the most hypotonic urine was not obtained until two hours after the completion of the infusion. In contrast, with the same subjects sitting, hypotonic urine was not elaborated during even longer periods of time, but was secreted one-half to one hour after recumbency was assumed.

The variation in endogenous creatinine clearance in some experiments may be explained in part by incomplete bladder emptying at low rates of urine flow. Although a rather consistent trend to lower clearance values may be noted in the periods during or just after the intravenous infusion, the significance of this is uncertain. Since, in these observations, concentration of urinary solutes was the variable under study and not their rate of output, and since the change under consideration was a fall of tonicity of the urine, collection and timing errors could only serve to delay the recognition of

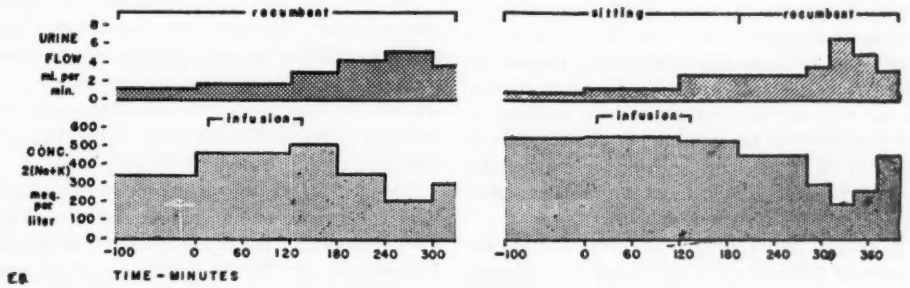

FIG. 3. PATIENT E. B.

Legend as in Figure 1.

such a fall in tonicity or decrease its apparent magnitude. Although more accuracy could have been achieved by means of an indwelling catheter, the presence of such a foreign body in the urethra for a period of many hours would not be conducive to minimum emotional stimulation of the supra-optico-hypophyseal system.

DISCUSSION

In each of the six experiments described above, during or following the infusion of 3 liters of an 0.9 per cent solution of NaCl, the urine volume rose sharply and the concentration of both Na and Cl fell to *below* their respective concentrations in the extracellular water as well as *below* their concentrations in the infusion solution, while the urinary specific gravity measured at 15° C. fell to or below 1.005 in the sitting and 1.003 in the recumbency studies. The concentration of urea plus ammonia concomitantly fell to less than one-fourth of the pre-infusion concentration in each instance. This excretion of water in excess of solutes is characteristic of the diuresis which follows the ingestion of water, of alcohol (17), of exposure to cold (19), and damage to the supra-optico-hypophyseal system (1). It may be inhibited in these instances by the administration of pitressin. In the experiments reported above the duration of the diuresis was limited, presumably because of stimulation of the supra-optico-hypophyseal mechanism by the increasing tonicity of the plasma and extracellular fluid which must of necessity have occurred since during the diuresis water was excreted in excess of solutes.

Murphy has observed that subjects receiving 20–30 ml./kg. of 0.85 per cent NaCl intravenously in 25–40 minutes have an initial water diuresis which he suggested was "related to dilution of circulating pitressin and related renal tubular effects" (29). In view of the fact that such dilution in our experiments should have been maximal at the conclusion of the infusion but diuresis did not occur in the sitting subjects until they assumed the recumbent position, sometimes several hours later, this hypothesis receives no support.

Welt and Orloff (30) have found that the intravenous administration of 4–6 per cent albumin in normal salt solution leads, in semi-recumbent subjects, to a water diuresis of as great or greater magnitude than that encountered in the studies reported here. Thus their observations indicate that it is not the dilution of the plasma proteins which is involved in the response. It is of interest that when, as a control for the albumin in saline experiments, they administered 2,500 ml. of saline alone intravenously there was no clear-cut water diuresis, although during the first hour of the infusion the urinary concentration of sodium chloride fell to 100 meq./l., and rose only to 145 meq./l. during the second hour. The difference between these findings and those reported here may be due to the fact that their subject received no fluids for 12–14 hours before the infusion and that he was semi-recumbent on a couch rather than flat on a bed.

Since the water diuresis produced by 4 per cent albumin in saline could be inhibited by adding 200 milli-units of posterior pituitary hormone to the infusion, Welt and Orloff suggested that the diuresis was produced by suppression of posterior pituitary activity.

Our observations, demonstrating that the isotonic expansion of extracellular fluid volume (both intra- and extravascular) was ineffective under the conditions of study in producing a water diuresis in the sitting subject, but did so during recumbency, suggest that the receptors for expanded volume (or an associated factor) may have their locus in the cephalad portion of the body. Since the supra-optico-hypophyseal system is believed to be largely involved in the control of water excretion and since it is so located, it may be suggested that the receptor cells, known to respond to changes in osmotic pressure, may also respond to hydrostatic pressure.

The failure of Lewis and associates (31) to secure consistent changes in the rate of urine flow by compression of the neck with an inflated blood pressure cuff, a maneuver which presumably increased the intracranial pressure, may well have been due to the antidiuretic effect of the considerable discomfort produced by the procedure.

The fact that an isotonic expansion of extracellular volume of some magnitude did not initiate a water diuresis in the sitting position is of interest in connection with the observation recorded for the fore-period here which has been repeatedly made in other studies (32–37) that the normally hy-

drated subject has an increased water diuresis upon lying down, with a decrease in urinary electrolyte concentration. This water diuresis, presumably due to decreased ADH action, differs from the comparable increase in urine volume encountered in the water-loaded subject, in whom ADH activity is presumably already minimal and whose excretion and *concentration* of electrolyte both rise upon assuming the recumbent position (37). The enhanced excretion of both water and electrolyte which occurs in edematous states associated with both heart and liver disease when the patient assumes a more recumbent position (38) may well be due in part to the redistribution of the already isotonically expanded extracellular volume.

CONCLUSIONS

1. Antidiuretic activity, ordinarily diminished by hypotonicity of the extracellular fluid, may also be diminished in the recumbent subject by isotonic expansion of the extracellular fluid volume.

2. The fact that an equal expansion of extracellular volume in the sitting subject does not significantly diminish antidiuretic activity, although there is an equal or greater expansion of both plasma and extracellular volume, suggests that the distribution as well as the magnitude of the expanded extracellular volume is of importance.

ACKNOWLEDGMENTS

The technical assistance of Mrs. Eleanor Harkins and Miss Miriam Halpin is gratefully acknowledged.

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Since this manuscript was submitted for publication, G. Blomhert has published a doctoral thesis (Over de zogenaamde waterdiurese, Scheltema & Holkema, Amsterdam, 1951) in which he describes the production of a "water-diuresis" in recumbent subjects receiving isotonic saline either by mouth or by vein.

CONCENTRATION OF SERUM PROTEIN-BOUND IODINE IN NORMAL MEN [1, 2]

By ROBERT G. TUCKER [3] AND ANCEL KEYS

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(Submitted for publication January 17, 1951; accepted June 4, 1951)

During recent years the determination of the protein-bound iodine (PBI) in the serum or plasma has been used with increasing frequency as a diagnostic aid. Various limits have been suggested for the serum PBI concentration in euthyroidism. The range most frequently recorded in the literature is from 4 to 8 gamma per cent of PBI. These values are not fiducial expressions of full statistical analyses of adequate numbers of persons; they are based on observations made by several groups of workers on limited numbers of persons judged to be euthyroid but otherwise not fully characterized (Table I). The present paper

TABLE I

Literature values for normal serum PBI in man
All values in micrograms per 100 ml. of serum.

Authors	N	Mean	S. D.	Range
Salter, Bassett, and Sappington (1)	21	5.7*	1.0*	4.0–8.0
Talbot and associates (2)	11	7.0		6.0–8.4
Perry and Cosgrove (3)	34	5.9	1.3	4.0–9.3
Conner and associates (4)		4.8		4.0–6.0
Kydd, Man, and Peters (5)	83	5.3†	1.0†	3.8–8.5
Starr and associates (6)	100	5.6	0.9†	4.0–8.5

* Calculated from Figure 2.
† Calculated from Figure 1.

reports the results, including statistical analysis, from 402 young and middle-aged men who are clinically "normal" in all respects and who represent active urban "white collar" workers in the upper Midwest.

[1] Abridgement of material contained in a thesis submitted by Robert G. Tucker to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] The subjects studied in the Laboratory of Physiological Hygiene were available in connection with a long-range research project sponsored by the U. S. Public Health Service on the recommendation of the Cardiovascular Study Section.

[3] Present adress: Radioisotope Unit, Veterans Administration Hospital, Nashville, Tenn.

METHODS

Serum PBI concentration was determined by the method of Barker (7) modified as follows: (*1*) In the distillation of iodine, sodium arsenite solution was substituted for sodium sulfite solution as the alkaline reducing substance in the trap. This modification was suggested by Dr. Barker in a personal communication. To 20 ml. of 5 *N* NaOH, 1.5 grams of As_2O_3 are added, and the resulting solution is diluted with distilled water to 100 ml. One-half ml. of this solution is used in the trap in place of 0.5 ml. of sodium sulfite solution. Aeration is no longer necessary. The cooled distillate and washings can be diluted to 25 ml., mixed, and the iodide content determined directly by cerate colorimetry. (*2*) Phosphorous acid was found to be the reagent which was most apt to cause difficulty. An aqueous 50 per cent solution of phosphorous acid (Fisher Scientific Company) was filtered through an asbestos mat and boiled with 2 ml. of Superoxol for one hour at approximately constant volume. This effectively removed traces of iodine and organic matter. (*3*) Blood serum was used for analysis rather than blood plasma. (*4*) Blank values were determined from completely inorganic samples.

The PBI method was studied and standardized with great care before any of the material reported here was analyzed. Known amounts of iodine as sodium iodide were added to serum in repeated recovery experiments which demonstrated apparent complete recovery of iodine with an occasional high value. For example, in the last series of such tests, 0.1 γ of iodine was added to each of four aliquots of pooled serum which had on repeated analysis an average of 4.75 γ of PBI per 100 ml.; the apparent recoveries of added iodine were 100, 100, 100 and 130 per cent. Three samples of serum were exchanged with the Department of Biochemistry of the Mayo Clinic with the results shown in Table II; both of the laboratories used the same analytical procedure.

Finally, the reproducibility of single measurements was checked. In a series of 12 aliquots, with a mean value

TABLE II

Collaborative check samples
Values for micrograms of iodine per 100 ml. of serum.

Sample	Test	Mayo Clinic	U. of Minn.
Human serum	Total I	3.6	4.3
Human serum	PBI	5.5	5.6
Human serum (exophthalmic goiter)	PBI	16.2	16.4

of 4.77 γ per 100 ml. of serum, the standard deviation was $\pm$ 0.60. This suggests that in nine out of 10 cases a single measurement of PBI will be within $\pm$ 1.0 γ per 100 ml. of the true (mean) value. It seems possible, however, that the error of measurement is not absolute but is related to the concentration of PBI in the sample. If the error were strictly proportional to the total concentration, we might estimate that in nine out of 10 cases a single PBI measurement will be within $\pm$ 21 per cent of the true (mean) value of the serum examined. From trials with high PBI sera, however, this appears to be an overestimate of the error and we conclude that for ordinary clinical work the reliability of a single measurement can be taken to be within 1 γ per cent.

The serum total cholesterol determination was made by application of the Liebermann-Burchard reaction to a Bloor extract. This procedure as used here at constant temperature, with cholesterol standards for every batch, closely checks with the Schoenheimer-Sperry digitonin method which was, in fact, applied in parallel from time to time (8). The basal oxygen consumption was determined with the Sanborn apparatus by the standard technique used by Boothby, Berkson, and Dunn (9). The normal weight for each subject was estimated according to his height and age by referring to the tables published from the Medico-Actuarial Investigations of 1912; these are sometimes called Davenport's tables and are, in essence, the tables almost universally used in the United States. The per cent of normal weight was calculated according to the formula: $\frac{\text{actual weight}}{\text{normal weight}} \times 100 = \text{per cent}$ of normal weight.

MATERIAL

The normal men used in the present work have been studied rather intensively in other respects (8, 10). They are of northern European ancestry (chiefly descended from stock from the British Isles) and are actively and successfully engaged in non-manual pursuits in the metropolitan communities of Minneapolis and St. Paul. Included here were 130 younger men (18 to 25 years of age) students in the University of Minnesota and 272 middle-aged (45 to 56 years of age) business and professional men. Each man was subjected to a physical examination by a competent internist and was carefully questioned as to medical history, fatigue, "nervousness," etc. The absence of complaints, tremor or abnormal heart rate and the presence of normal basal metabolic rate, all contributed to the considered judgment that these men were euthyroid. This judgment was sustained by repeated examinations in three successive years. All of the men came to the laboratory in the morning without breakfast, and all data refer essentially to the basal state.

RESULTS

The descriptive statistics for the sample of normal men are given in Table III. The sample of normal serum PBI value did not form a normal frequency distribution curve (Figure 1). By calculation of the beta coefficients the distribution was found to be significantly skewed and peaked so that the standard deviation is an inappropriate

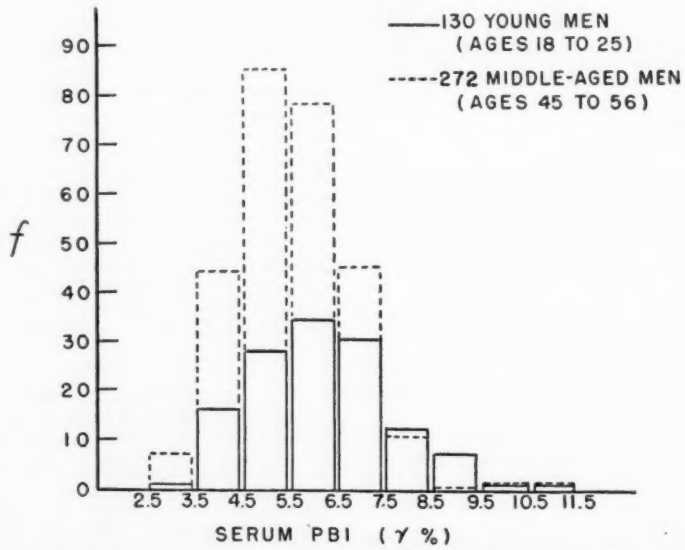

FIG. 1. FREQUENCY DISTRIBUTION OF SERUM PBI VALUES IN NORMAL MEN

TABLE III

Descriptive statistics for the sample of normal men

	Statistic	PBI	BMR	Cholesterol
		γ %	*ml. O_2/m.²*	*mg. %*
Young men (18–25 years; N = 130)	Mean	6.24	128.2	188.6
	S. D.	1.46	11.7	36.3
	Range	3.1–11.0	78.8–171.6	106–285
Middle-aged men (45–56 years; N = 272)	Mean	5.63	116.3	261.3
	S. D.	1.21	11.6	45.7
	Range	2.6–11.1	77.3–161.6	157–421
Combined groups (N = 402)	Mean	5.83	120.2	237.8
	S. D.	1.32	12.9	54.8
	Range	2.6–11.1	77.3–171.6	106–421

measure of dispersion. Therefore standards for the serum PBI concentration were established for the population from which the sample was drawn by computing the 1.0, 5.0, 95.0 and 99.0 percentile values. These standards are listed in Table IV which provides, in each group, the expected ranges, as upper and lower limits, for 95 and 99 per cent of the normal population. For example, in normal young men 95 per cent of observed values are expected to be greater than 3.98 gamma per cent of PBI, and 99 per cent greater than 3.58 gamma per cent; at the other extreme 95 per cent of the observed normal values are expected to be less than 8.70 gamma per cent of PBI, and 99 per cent less than 9.82 gamma per cent.

The relationships between the serum PBI concentration and several other characteristics of these men were studied by an analysis of variance. The independent variables considered were: (*1*) age; (*2*) the basal metabolism expressed as ml. of oxygen consumption per square meter of body surface per minute; (*3*) the serum total cholesterol concentration; (*4*) the per cent of normal body weight. The relationships between these characteristics and the PBI were studied for the sample as a whole and for the group of young men and the group of middle-aged men.

TABLE IV

Range of serum PBI concentration for 95 and for 99 per cent of the population of normal men

Values for micrograms of iodine per 100 ml. of serum

Group	N	Level	Lower limit	Upper limit
Young men (18–25 years)	130	95%	3.98	8.70
		99%	3.58	9.82
Middle-aged men (45–56 years)	272	95%	3.87	7.52
		99%	3.32	8.51
Combined groups	402	95%	3.90	8.15
		99%	3.35	9.55

No significant correlation was observed between age and the serum PBI within the limited ranges of either the young group or the middle-aged group. However, the mean for the young group of normal men was significantly higher (above the 1 per cent level), as shown by calculating Snedecor's F ratio, in serum PBI than the mean for the middle-aged group.

A slight but significant positive correlation was demonstrated for the combined groups between the serum PBI and the basal metabolic rate ($r = +0.113$). This was accepted with reservations, because it was shown that the relation approaches curvilinearity. No correlation was observed between the serum PBI and the basal metabolic rate within the young group or within the middle-aged group of men. Therefore, in normal men variations in the basal oxygen consumption are due largely to factors other than the circulating PBI.

In the middle-aged group of normal men a slight but significant, positive, and linear correlation ($r = +0.163$) was observed between the serum PBI and the serum cholesterol concentration; no such relation was found in the group of normal young men. For the gross sample of normal men a curvilinear relationship was demonstrated between the serum PBI and the serum cholesterol.

No relationship was demonstrated between the serum PBI concentration and the per cent of normal body weight in normal young men, in normal middle-aged men, or in the gross sample.

DISCUSSION

The serum PBI data for the sample of men reported here constitute the largest sample of normal men which has been studied with modern analytical techniques. The sample is by no means random. The subjects were selected with respect to sex, race, age, economic status, geographic habitat and physical condition. Nevertheless, the frequency distribution of the 402 serum PBI values is remarkably similar to the sample described by Kydd, Man, and Peters (5). Our mean value of 5.8 gamma per cent of PBI is significantly higher than the mean of 5.3 gamma per cent calculated from Kydd's data. However, by using a Chi

square test for goodness of fit, and by making the two distributions comparable by expressing them in units of absolute deviation from their respective absolute means, the obtained Chi square value of 1.83 associated with seven degrees of freedom indicates that a discrepancy as large as that observed could occur through chance alone 97 times out of 100. From this very close relationship we infer that there is no statistical difference between the shape of the two frequency distribution curves, and that the distribution of PBI values in the common population from which they were drawn is non-Gaussian. The two samples would be identical in all respects except for the apparent presence of a systematic analytical difference which could be explained, possibly, as a result of slight differences in method or of a difference in the age composition of the samples.

With respect to age our results are in agreement with those of Kountz, Chieffi, and Kirk (11) who reported, "a gradual decrease in the organic serum iodine concentration . . . with advancing years." The resemblance to the basal metabolic rate trend with age may be noted, but the age trend in serum PBI concentration is considerably less pronounced. The lack of correlation between the serum PBI concentration and the basal oxygen consumption merely emphasizes that the one is not closely dependent on the other in this normal range.

Our data on cholesterol are somewhat difficult to interpret. However, we are in substantial agreement with Peters and Man (12) who found no important relationship between the precipitable iodine and the cholesterol in the serum in euthyroidism. There is no denying the fact that a very slight relation does exist between the serum PBI and the cholesterol level in the middle-aged men, however.

Williams (13) "concluded that an alteration of thyroid function does not have much etiologic significance in obesity." This view is confirmed by the present study. In 33 obese men of the Minnesota study (those whose body weight exceeded 126 per cent of the normal mean standard weight), only three had serum PBI values of less than 4.0 gamma per cent. This suggests that in otherwise normal men obesity is due, for the greater part, to factors other than the activity of the thyroid gland.

SUMMARY

From the study of the protein-bound iodine (PBI) in the serum of 402 normal men in Minnesota, the following conclusions are drawn:

1. The frequency distribution of serum PBI values in normal men is significantly peaked and skewed.

2. Standards for serum PBI values have been established for the 95 and for 99 per cent of the population for which these normal men represent a sample.

3. Normal middle-aged men (45 to 56 years of age) are significantly lower in serum PBI than normal young men (18 to 25 years of age).

4. In normal men a small but significant, positive correlation ($r = + 0.113$) exists between the serum PBI and the basal oxygen consumption. Differences in the basal metabolic rate of normal men are dependent largely on factors other than the circulating PBI.

5. In normal middle-aged men (45 to 56 years of age) a positive, linear correlation ($r = + 0.163$) exists between the serum PBI and the serum total cholesterol concentration; no such relation obtains in normal young men (18 to 25 years of age). For both young and middle-aged normal men studied collectively, a curvilinear relation exists between the serum PBI and the serum cholesterol.

6. In normal young men and in normal middle-aged men, no relationship exists between the serum PBI and the relative obesity of the individual.

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PRECIPITABLE IODINE OF SERUM (SPI) IN DISORDERS OF THE LIVER [1]

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Recently, high values of precipitable iodine of the serum (SPI) in certain instances of hepatitis and variable values in cirrhosis of the liver have been reported from this laboratory (1, 2). Inasmuch as these sporadic high values in hepatitis were at variance with our usual findings, and because high, low and normal values have been found in cirrhosis, additional studies of patients with disorders of the liver have been conducted.

MATERIALS AND METHODS

Patients with obstructive jaundice, cirrhosis of the liver and infectious hepatitis were observed on the wards of the New Haven Hospital. All were in a post-absorptive state. None had had gall bladder dyes, diodrast, lipiodol or other substances known to give high artifactual values of SPI. None had had any mercurial diuretics within the period during which these substances may affect the determination of SPI (3). None had had any unusual contact with inorganic iodine (4). SPI, lipids, proteins, bilirubin, alkaline phosphatase and the percentage of bromsulphalein retention were determined by methods previously described (2, 5–7).

RESULTS

In obstructive jaundice six values of SPI obtained in five individuals were all within the established normal range (2) (Table I). In addition to considerable jaundice these patients had grossly disturbed liver function as measured by the high ratio of free to total cholesterol and other tests. In cirrhosis of the liver 31 values of SPI in 21 individuals varied from 1.0 to 8.8 gamma per cent (Table I). Of these values, however, only two are greater than occur in normal persons. A concentration of 8.0 gamma per cent, a value barely exceeding the normal 7.8 gamma per cent, was found in HD in whom a value of 6.6 gamma per cent had been found 16 months previously at a time when her clinical condition appeared to indicate a more decompensated cirrhosis than when the later result was obtained. A value of 8.8 gamma per cent was obtained in AS who had had symptoms of cirrhosis for only three months. A biopsy of the liver showed the typical histology associated with cirrhosis without evidence of other liver disease. Low values are more common. In fact, SPI may be as low as concentrations usually associated with hypothyroidism although other evidences of this disorder are absent. Despite the occurrence of these abnormal values, SPI is often normal in cirrhosis of varying severity.

In infectious hepatitis 18 values in nine patients varied from 3.4 to 11.5 gamma per cent (Table I). Serial observations of SPI during hepatitis show that early in the disease SPI is elevated but that as the disease continues, SPI falls to a normal value (Figure 1). All of the abnormal concentrations of SPI were obtained during the first four weeks of the illness. Subsequently, SPI remained normal despite the continued activity of the hepatitis as measured by the continued abnormalities of liver function, jaundice and often clinical condition of the patient. In M. D., for example, about two months before her death from hepatitis when 72 per cent of the serum cholesterol was not esterified and the other liver function tests were markedly deranged, SPI was 5.3 gamma per cent. However, at this time the disease had been present for more than four months. The highest SPI was obtained in RC who was examined 16 days after symptoms of the disease first appeared.

DISCUSSION

In cirrhosis of the liver, reduction in SPI is correlated with evidences of the severity of the disease, but not with any single functional test. Because of the variability of SPI within the normal range only abnormally low concentrations can be considered. All of these values are associated with

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TABLE I

SPI, lipids, and liver function tests in disorders of the liver

Patient	Onset	Date	SPI	Serum							Bromsulphalein retention in 45″
				Cholesterol			Protein		Bilirubin	Alkaline phosphatase	
				Total	Free	Free/Total	Albumin	Globulin			
			gamma %	*mg. %*	*mg. %*		%	%	*mg. %*	*Bodanski units*	%
Obstructive Jaundice											
A. L.		2–19–49	5.0	128	95	0.74	2.74	3.38	18.1	12.9	14
R. K.		2–27–50	4.8	315	213	0.68			10.6	48.6	
		3–4–50	5.5	290	160	0.55					
B. A.		7–5–50	6.3	246	152	0.62	2.43	3.16	6.5	36.6	
J. M.		1–18–49	6.8	163	108	0.66	3.32	2.70	19.4	48.8	
R. D.		3–7–51	6.2	463	265	0.60	3.20	5.20	9.6	44.9	
Cirrhosis of the Liver											
P. P.		1–19–48	1.0	70	56	0.62	0.92	5.09	15.4	8.4	
J. Q.		11–3–47	2.3	267	113	0.41	1.95	4.37	1.9	18.9	15
P. P. A.		6–14–48	3.0	170	68	0.40	1.64	4.92	2.9		50
E. B.		12–15–49	3.0	169	124	0.73	1.17	4.11	13.6	2.5	28
		12–20–49	3.3	156	117	0.75	1.29	4.90			
M. G.		4–21–47	3.3	116	47	0.41	1.82	3.60	1.3		36
R. C.		6–25–47	3.7	154	49	0.32	1.68	3.72			
M. M.		11–21–47	4.4	132	85	0.64	1.51	5.46	11.5	5.9	55
		12–11–47	3.7	108	60	0.55	2.52	3.89			
		1–7–48	4.1	152	87	0.56	1.79	5.14			
		5–21–48	4.3	149	83	0.55	1.89	5.12			
H. B.		5–5–47	4.0				1.67	3.38			
B. D.		2–1–50	5.0	140	97	0.69	1.61	4.97	3.6	7.9	
		2–13–50	4.0	177	138	0.78	1.33	5.11	5.5	12.7	
G. M.		4–17–47	4.2	163	81	0.50	1.33	5.84	3.5		43
M. L.		2–11–50	4.2	155	80	0.52	1.51	5.82	2.0	13.7	42
A. C.		4–5–48	4.8	196	63	0.32			0.7	17.0	22
F. B.		12–19–45	7.3	175	77	0.44					
		10–6–47	7.3	204	69	0.34					
		3–2–50	4.8	177	41	0.23					
M. S.		12–13–49	4.9				2.67	3.98	0.3	7.4	20
T. F.		1–21–49	5.7	255	129	0.51	2.04	3.69	4.6	18.5	22
		12–8–49	5.3	311	80	0.26	4.44	2.96	1.6	7.9	27
H. M.		3–29–50	5.6	212	70	0.33	1.39	3.98	3.3	18.4	39
A. M.		12–29–49	6.0	176	91	0.51	1.88	4.71	5.1	2.4	29
		1–9–50	6.0	176	75	0.42	3.49	4.70	5.2	7.8	33
F. C.		4–26–50	6.3	114	34	0.30	2.52	3.55	2.2	5.3	12
H. D.		11–2–48	6.6	143	40	0.28	3.58	2.90	1.6		27
		3–21–50	8.0	167	42	0.25	3.46	2.30			6
M. K.		2–11–50	7.3	199	51	0.26	3.88	3.20	3.2		
A. S.		1–23–50	8.8	209	62	0.30	2.32	4.23	0.9	7.3	19
Infectious Hepatitis											
J. M.	3–26–46	4–9–46	8.8	151	48	0.32	3.74	2.84			
J. H.	3–17–49	5–7–49	3.4	56	40	0.72	1.78	5.22			
G. S.	8–1–49	2–7–50	6.4	203	55	0.27	4.23	3.17	2.0	4.4	
M. D.	1–3–49	5–6–49	6.9	209	172	0.82	2.10	3.50	8.6	51.8	22
		5–11–49	5.3	154	116	0.75	2.10	3.50	18.9	38.4	
A. M.	5–20–50	6–5–50	11.1	150	131	0.87	2.01	3.37	24.9	19.1	
		6–8–50	9.3	147	130	0.89					
		6–13–50	9.8	193	155	0.80			12.7	14.3	
		6–19–50	7.5	206	101	0.49					
		6–21–50	6.3	218	83	0.38			4.3	5.9	
		7–3–50	7.2	229	74	0.32			3.8	6.2	
M. B.	3–7–50	3–23–50	10.0	139	65	0.46	3.77	3.61	8.8	10.8	
		3–29–50	6.6	167	44	0.26			4.3	11.5	42
		5–25–50	6.2	193	54	0.28			0.7	5.4	5
R. C.	3–18–50	4–3–50	11.5	185	127	0.69	3.40	2.37	5.1	13.7	
		4–6–50	9.4	214	89	0.42					
		4–13–50	7.1	235	36	0.15			2.1	8.8	
D. G.	12–8–50	12–21–50	8.8	263	213	0.81	3.52	3.20	9.9	19.8	61

a high percentage of nonesterified cholesterol in the serum, a sensitive indication of liver damage (8). However, such lipid disturbances or alterations in the concentration of cholesterol are not always accompanied by reduction in SPI. Correlation with the other liver function tests is even less apparent. That the low values of SPI in cirrhosis might be related to the frequent occurrence of a decreased amount of albumin in the serum of patients with this disease has been suggested (1) inasmuch as the organic or thyroxine-like fraction of the total iodine of the serum is attached to the serum proteins (9, 10). However, if SPI be compared directly with the concentration of albumin no definite correlation is found. The lowest values of SPI are found in patients with diminished serum albumin, but SPI is often moderately, or not at all, reduced in other patients with similar reductions of albumin. Comparable abnormalities of serum albumin and SPI are seen in nephrosis without evidence of hypothyroidism and without any exact correlation with each other (1, 2). The cause of the low values of SPI in both conditions is unknown. In cirrhosis of the liver reduction in the serum albumin is associated with malnutrition (11). However, SPI is not abnormal in patients with marked malnutrition from various causes (12). In nephrosis and cirrhosis abnormalities in the proportions of the two components of the albumin fraction have been found by conducting the electrophoresis at pH 4.0 (13). Such possible qualitative alterations in the serum albumin may affect the usual attachment of thyroxine to the proteins.

In contrast to the low values of SPI found in cirrhosis, high values are found early in the course of hepatitis. Many of the values are within the range usually associated with hyperthyroidism. However, although other objective determinations of thyroid function such as the measurement of the basal metabolic rate or the lipids of the serum are unreliable in acute hepatitis, none of these patients had clinical evidences of hyperthyroidism nor was the course suggestive of this disease. High values of SPI are found also in pregnancy (14), but the cause of the rise in the two conditions seems to be dissimilar inasmuch as the rise occurs in hepatitis only early in the course and is not related to the severity of the disease.

Only a small proportion of the iodine in urine resembles thyroxine either in euthyroid persons or hyperthyroid patients, whereas after the administration of thyroxine appreciable amounts are found in the feces (15, 16). Administered thyroxine soon leaves the plasma and appears in the liver, muscle and other tissues (17). In the liver not only does a steady degradation of the thyroxine occur, but also a proportion of the thyroxine is found in the bile and finally in the intestinal tract. When larger amounts of thyroxine are given, the proportion of thyroxine-like iodine found in the feces becomes high and, if the bile duct be ligated, the labeled thyroxine leaves the plasma much more slowly (15). That this degradation and excretion of thyroxine by the liver is important is suggested by a greater effectiveness of administered thyroxine when the liver is partially removed (18). Also thyroxine given intraperitoneally is less effective

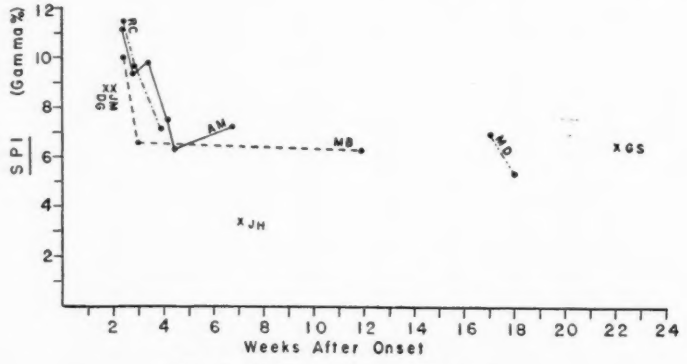

FIG. 1. SPI IN INFECTIOUS HEPATITIS

than when given subcutaneously unless the bile duct is ligated (19).

In instances of biliary obstruction studied the excretory function of the liver is destroyed, but the liver cells may not all be sufficiently damaged to prevent the degradation to inorganic iodine of whatever thyroxine is presented. The inorganic iodide is excreted by the kidney and a normal SPI is found even though considerable derangement of hepatic function may be present. The normal SPI in obstructive jaundice indicates that the high SPI found early in hepatitis is not referable simply to retention.

In hepatitis, tests of liver function are found to be abnormal early in the course of the disease (8, 20, 21). Biopsies of the liver taken during hepatitis and autopsy studies in fulminant cases show that widespread damage of the hepatic cells occurs soon after the onset (22–24). After a latent period, areas of regeneration appear and extend even though the patient may remain desperately or even fatally ill (24). The initial widespread damage caused by the disease may be sufficient to prevent the normal destruction of thyroxine by the liver. In addition, at this stage of the disease, the excretory function of the liver is destroyed. The kidney is able to excrete largely inorganic iodide and the organic iodinated compounds may, under these circumstances, increase in the serum. Later in the disease a sufficient number of liver cells may either recover or regenerate; a normal SPI is then found despite the continuance of other evidences of gross hepatic abnormalities.

SUMMARY AND CONCLUSIONS

1. SPI in five patients with obstructive jaundice was normal.

2. In cirrhosis SPI was either normal or low. When low, the serum albumin may also have been low but there was no consistent relationship between the two. The depressed SPI may be correlated with the severity of the disease but with no single test of hepatic function.

3. In hepatitis, elevation of SPI occurred early in the disease. After the first four weeks of illness no abnormal values were found. The possible significance of these alterations has been discussed.

ACKNOWLEDGMENT

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POOL SIZE, TURNOVER RATE, AND RAPIDITY OF EQUILIBRATION OF INJECTED ISOTOPIC URIC ACID IN NORMAL AND PATHOLOGICAL SUBJECTS [1, 2]

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Isotopically labeled uric acid has been injected intravenously into normal subjects and subjects with various diseases in order to determine the pool size and turnover rate of body uric acid. Such studies have been carried out both in this laboratory and in other laboratories (1, 2). It is the purpose of this communication to examine some of the assumptions used in these calculations and to present data which show that these assumptions are not always valid. The disappearance curves of urinary isotopic uric acid for several subjects and several therapeutic regimens will be discussed.

EXPERIMENTAL SUBJECTS

Normal controls with normal or elevated serum urate concentrations and patients with recognized clinical maladies served as the subjects. The patients were suffering from gout, acute gouty arthritis, rheumatoid arthritis, polycythemia vera or chronic myelogenous leukemia. The normal and hyperuricemic subjects were ambulatory. With but one exception all the other patients were hospitalized, the majority in a metabolism ward. In most instances the experimental subjects were on a constant diet of low purine content which was served from the diet kitchen. The fluid intake was maintained relatively constant but not precisely so. An abstract of the clinical history and physical examination of each subject is given in the protocols.

CHEMICAL PROCEDURES

The isotopically labeled uric acid employed in these studies was synthesized by the method of Cavalieri, Blair, and Brown (3). It contained N^{15} in the 1 and 3 positions and had an overall isotope concentration of approximately 16 atom per cent excess. In preparing a sample of the isotopic uric acid for intravenous injection, a quantity (25–100 mg.) of the purified uric acid was rinsed off a tared weighing glass with absolute alcohol into a previously autoclaved wide mouth glass jar. A few drops of alcoholic phenol red indicator were added. The jar was placed in an oven at approximately 70° C. until the alcohol had evaporated, and a previously autoclaved plastic top was screwed onto the jar. At the time of the injection the uric acid was dissolved in 25–50 ml. of sterile (autoclaved) sodium hydroxide. Concentrated hydrochloric acid followed by dilute hydrochloric acid was added dropwise with stirring until the yellow color of the indicator appeared. The solution was immediately injected intravenously and the syringe rinsed several times through a three way stopcock with small volumes of isotonic saline solution. The neutral solution of uric acid was not allowed to stand more than five to ten minutes because of the tendency for uric acid to precipitate.

The sodium hydroxide solution was prepared in such a concentration that when neutralized with hydrochloric acid the resulting solution was isotonic in sodium chloride. Uric acid solutions prepared in this manner were found to be sterile and quantitatively accurate. No untoward clinical effects have thus far resulted from the injection of such a solution.

Urine was collected in four or six hour periods or at each voiding (*ad libitum*) for the first and second day of each experiment. Thereafter several samples were combined before processing.

Uric acid was isolated from fresh urine by precipitation with ammonium chloride and ammonium hydroxide (4). The precipitated ammonium urate was washed several times with ammonium sulfate-ammonium hydroxide wash solution and the drained precipitate was dissolved in concentrated sulfuric acid, the container being kept cool by immersion in a dry ice slurry. Distilled water was added and the uric acid that precipitated was centrifuged down. After the supernatant liquid was decanted, the uric acid was dissolved in sodium hydroxide. Norite-A was added, and the tube was chilled in the cold bath. The sodium urate solution was filtered through Whatman No. 3 paper by gravity. The filtrate was acidified with concentrated hydrochloric acid and the reprecipitated uric acid was centrifuged down. These processes were repeated until a white product was obtained. The uric acid was finally suspended several times in absolute alcohol and filtered by suction on Whatman No. 50 paper. Aliquots

[1] These studies were aided in part by a Grant-in-Aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council and by a grant from the John A. Hartford Foundation, New York City.

[2] This paper includes data which may be submitted by William Garner to the Graduate School of the University of Buffalo in partial fulfillment of the requirements for the Master's Degree in Biochemistry.

of the purified uric acid samples were converted to ammonia by the Kjeldahl procedure according to the modification of Hiller, Plazin, and Van Slyke (5) and the ammonia converted to nitrogen by the hypobromite method of Sprinson and Rittenberg (6). The determinations of N^{15} were made on a Consolidated-Nier Isotope-Ratio Mass Spectrometer (Model 21-201).

The nitrogen content and the extinction coefficient at 290 mμ of random samples of the isolated uric acid checked similar values of carefully purified reagent uric acid.

EXPERIMENTAL DRUGS

The three anti-rheumatic drugs used in these studies were colchicine, cortisone (Kendall's Compound E) and ACTH (adrenocorticotropic hormone). The general procedure was to inject isotopic uric acid and collect urine samples for approximately one week. A drug was then administered for a period not exceeding three days. Isotopic uric acid was again injected.

Colchicine was given in hourly oral doses of 0.5 mg. until gastrointestinal distress developed. Cortisone or ACTH was administered twice daily in 50 mg. intramuscular injections. In some subjects the administration of cortisone or ACTH was continued through the experimental period. Any alteration in the size of the metabolic pool or velocity of turnover in the experiments following drug therapy must be interpreted in relation to acute experiments and does not presume to predict what might have happened if the drugs had been continued for a longer period of time and the pool determined at a later portion of the experiment. The experimental data from some of the control phases of the therapeutic periods were discarded because of technical discrepancies.

THEORY AND ASSUMPTIONS

In order to develop the simplest case concerning the behavior of injected isotopic uric acid, the assumptions enumerated below will be made tentatively. It is quite probable that none of the assumptions will be completely justified subsequently by experimental data but if the mathematical relationship finally derived tends to coincide with the observed findings, the assumptions will acquire some validity. Let us assume that:

(*1*) All the uric acid in the body is freely diffusible and at the same concentration. This total amount of uric acid will be identified as the *body pool* of uric acid.

(*2*) The size of this body pool (in milligrams or in millimols of uric acid) is homeostatically fixed (constant). This is equivalent to saying that the rates of origin and disposal are equal.

(*3*) Intravenously injected, isotopically labeled uric acid mixes completely and immediately with all the uric acid in the body pool.

(*4*) The molecules of isotopic uric acid are indistinguishable from the naturally occurring molecules insofar as metabolic processes or excretion are concerned.

(*5*) The uric acid synthesized[3] by the body contains no N^{15} in excess of normal abundance. No catabolic fragments of enriched uric acid are reused for uric acid synthesis. Furthermore, no isotopic uric acid escapes from the body pool and re-enters this body pool at a later time after the isotope concentration of the pool has changed.

(*6*) The concentration of isotope in the uric acid collected in the urine during a particular period is the same as the concentration of isotope in the uric acid of the body pool during that period.

(*7*) Uric acid is excreted in the urine at a constant rate. If this is not correct, then urine must be collected in short intervals. Otherwise uric acid collected during part of an interval will be diluted during the rest of the interval with a greater or lesser amount of uric acid of a different isotope concentration. To be precisely evaluated this would require the integration of the excretion curve. In practice, the excretion is assumed to be sufficiently constant, and the isotope concentration of an interval is placed at the midpoint of the interval. Of course, if uric acid excretion were constant only on a 24 hour basis, choosing the intervals as 24 hours might tend to smooth out the diurnal variations.

If all these assumptions are made then the equation describing the fall of the isotope concentration of urinary uric acid with time will be:

$$-\frac{dI}{dt} = kI.$$

This is because the probability of excreting isotopic molecules is directly proportional to their concentration in the mixture of isotopic and nonisotopic uric acid. As more isotopic molecules are removed, however, they are replaced by nonisotopic molecules (from synthetic processes) and the probability of withdrawing labeled molecules is decreased.

The differential equation integrates to the following:

$$\ln I = kt + c.$$

Thus when the logarithm of the isotope concentration of the urinary uric acid is plotted against time, a straight line should result.

By rearrangement of the fundamental differential equation it can be seen that:

$$k = \frac{-\frac{dI}{dt}}{I}.$$

In other words, k is the rate of change with respect to time of the isotope concentration, in terms of the prevailing isotope concentration. This is identified as the turnover rate constant and has the dimensions, reciprocal days. In the present situation it is equivalent to the turnover rate expressed as pools per day.

When $t = o$, the integrated form of the equation can be solved for c (or the curve can be graphically extrapo-

[3] The word "synthesis" as here used means the formation of uric acid from any source whatsoever.

lated back to t = o). The antilogarithm of this value can then be substituted into the isotope dilution equation to calculate the pool size at t = o or immediately after the injection of the labeled uric acid:

$$A = a\left(\frac{I_i}{I_0} - 1\right)$$

A = Pool size (in same units as a).
a = Amount of injected uric acid.
I_i = Isotope concentration of injected uric acid.
I_0 = Isotope concentration of body uric acid immediately after mixing with injected uric acid.

DISCUSSION OF POOL SIZE AND TURNOVER RATE

Benedict, Forsham and Stetten (1) have employed the aforementioned equations to calculate pool size and turnover rate on three normal subjects and two gouty subjects. The normal pool size ranged from 1,145 to 1,341 mg. with turnover rates ranging from .533 to .757 pools per day. Their gouty subjects had pool sizes of 4,742 and 18,450 mg. and turnover rates of .524 and .463 pools per day, respectively.

Geren and coworkers (2) injected isotopic uric acid into a normal subject and determined the pool size to be 944 mg. with a turnover rate of .83 pools per day.

In this laboratory a number of human subjects has been injected intravenously with N^{15} labeled uric acid. The results of 26 experiments are presented in Figure 1. The common logarithm of the isotope concentration of the urinary uric acid has been plotted against the time in days. It is apparent that the curves are essentially straight lines as had been predicted from the basic equations and assumptions. In some of the experiments, however, there is a tendency for one or more of the initial points to lie above the best straight line drawn through the latter points. This phenomenon has revealed itself in the present work probably because of the frequency of sampling during the initial periods of the experiments. Furthermore, not every subject shows it so that several subjects must be studied before it need appear. Some se-

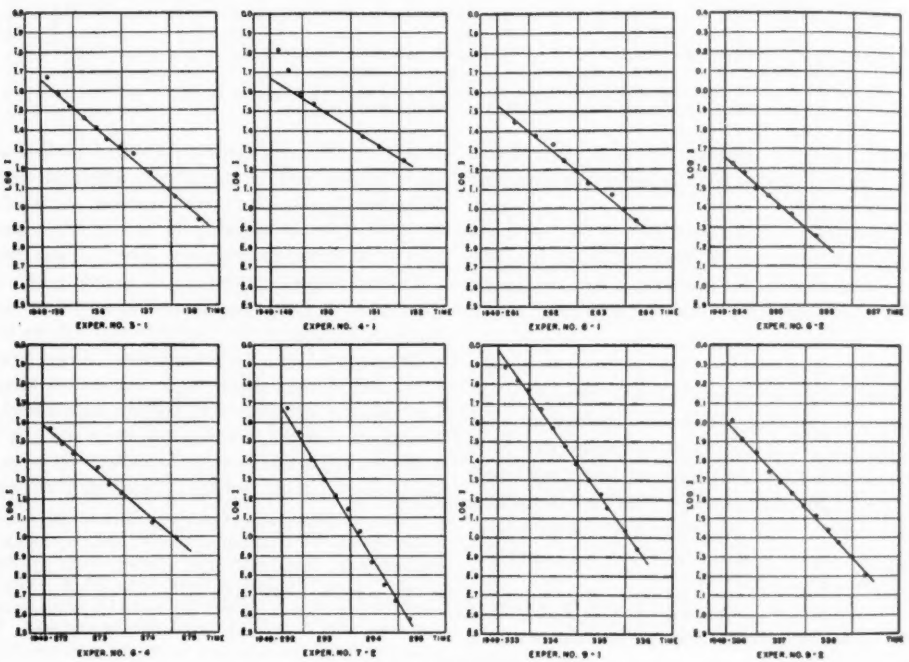

FIG. 1.

(See also Figure 1, *Continued*)

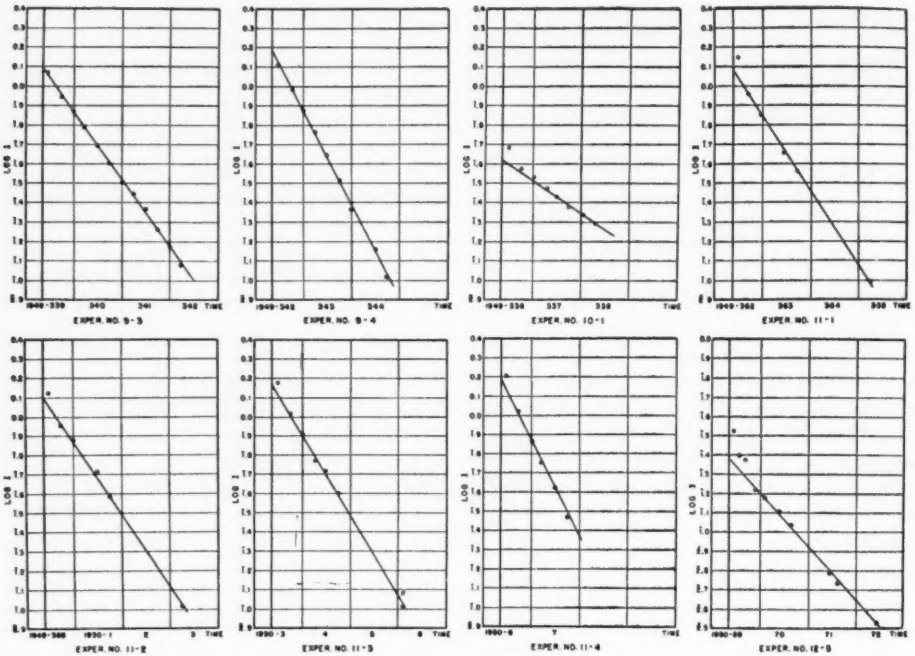

FIG. 1. CONTINUED

lected curves of Benedict, Forsham, and Stetten (1) are slightly concave, suggesting the same phenomenon.

Calculation of the pool size is dependent upon determining the isotope concentration of the body pool immediately after injection of the isotopic uric acid since only at this time is the quantity of isotopic uric acid in the body accurately known. At a later time this amount will be decreased by any amount catabolized and excreted. Since it is not analytically feasible to determine the concentration of isotopic uric acid in the body immediately after the injection, one does the next best thing which is to extrapolate successive values back to the time of injection. This procedure has another advantage in that momentary irregularities in equilibration are minimized.

Extrapolation of the linear curves in Figure 1 can be performed easily, but extrapolation of the non-linear curves presents some difficulties. If the injected isotopic uric acid were assumed to be slowly equilibrating during the initial periods of these studies and if no isotopic uric acid were being lost by catabolism and excretion, one would be justified in extrapolating the latter straight portion of the curves to zero time. The pool size thus calculated might be called the "eventual pool" to distinguish it from the "immediate pool" or the amount of uric acid which equilibrates with the injected material within a few minutes. Since, however, it is obvious that isotopic uric acid is being continuously excreted and perhaps catabolized,[4] it appears that the so-called "eventual pool" is a mathematical artifact except in those cases where the "eventual pool" and the "immediate pool" are

[4] Folin, Berglund, and Derick (7) were unable to recover as urinary uric acid all of the uric acid they injected intravenously, thus suggesting another route of disposal. Stetten occasionally found some N^{15} in urinary urea after injecting N^{15} labeled uric acid (1). Presumably this could only arise by the catabolism of uric acid. It should be noted, however, that Geren and associates (2) recovered nearly quantitatively the N^{15} uric acid injected intravenously in one study on one subject.

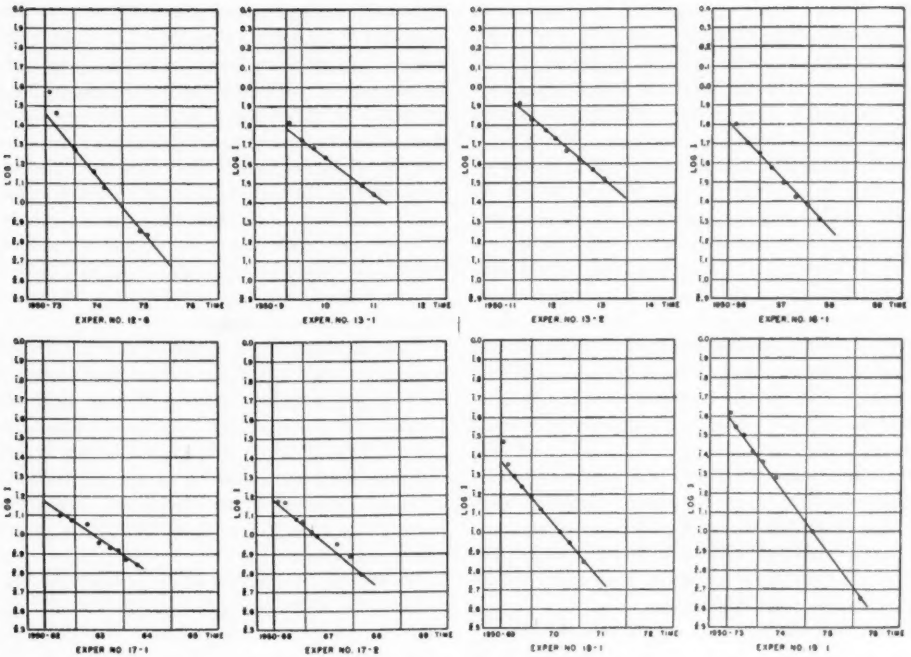

FIG. 1. CONTINUED

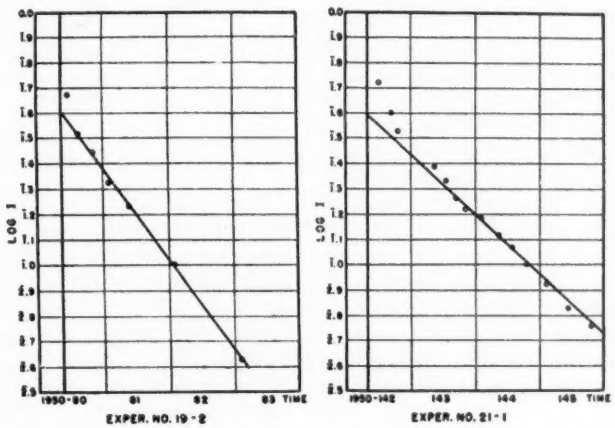

FIG. 1. CONTINUED

identical, *i.e.*, where the logarithmic curve is a straight line.

One method of calculating the pool size at any time involves a consideration of the amount of isotopic uric acid remaining in the body at that time with respect to the isotope concentration of uric acid in the body pool at that time. However, it is difficult to estimate the amount of uric acid remaining in the body at any time since this involves calculating the quantity of isotopic uric acid which has been disposed of prior to time t. The calculation of the amount of labeled uric acid excreted as uric acid is usually based upon an inaccurate colorimetric method. Furthermore, there is the evidence already cited which suggests disposal of labeled uric acid by a catabolic pathway, although it is difficult to assess the magnitude of this process. It would be desirable if one could calculate the total amount of body uric acid with which injected labeled uric acid would eventually equilibrate because in the gouty subject, at least, it is usual to find uric acid widely distributed and in forms which might not equilibrate rapidly. The data, available here or indeed anywhere, are inadequate for such calculations.

The "immediate pool," or the amount of body uric acid that equilibrates with injected uric acid, can be calculated from the extrapolated value of ln I at $t = o$. If the logarithmic curve is straight, this extrapolation is simple. If the logarithmic curve is not straight, its prolongation to the y axis, especially if accomplished free hand, may result in a considerable error because of the nature of the logarithmic units in this region. It has been found empirically that if one considers that a second but brief logarithmic process is superimposed upon the more stable disappearance process, the resultant of these two curves is a curve that closely resembles the observed curve. These two logarithmic straight lines can be extrapolated quite accurately and their y intercepts combined to approximate the intercept of the original curve. In practice this calculation can be carried out as in Figure 2. The common logarithms of the original points were plotted against time, producing a curve which was straight in the latter portion. This straight portion was extrapolated to zero time. The antilogarithm of each point on the original curve had subtracted from it the antilogarithm of the corresponding point on the extrapolated curve. When the logarithms of these differences were replotted at the proper time intervals, an approximately straight line lying below the other two curves was derived. The antilogarithms of the y intercepts of the two straight lines were added to give an estimate of the antilogarithm of the y intercept of the original curve. This estimate was then used in the isotope dilution equation to calculate pool size. Although the foregoing calculation is based upon a second additive logarithmic process, it should be emphasized that this method of approximation is only empirical and at present no interpretation is implied.

The turnover rate as calculated from the straight portion of the logarithmic curves was presumed to represent a definite concept so long as it was expressed in pools per day. Any attempt to multiply this value by the pool size in milligrams or millimols is valid only if both pool size and turnover rates are measured at the same time. This is obviously not true if the logarithmic curve is not linear near $t = o$.

It may be concluded that in general the pool size can be estimated only immediately after the injection of the labeled uric acid and that even then caution is necessary in extrapolating the isotope disappearance curve. The turnover rate in pools per day is probably valid, but if expressed in milligrams or millimols of uric acid per day it is only valid if the equilibration of injected uric acid with body uric acid occurs immediately.

RESULTS

Disturbance of uric acid metabolism and implications of therapy

Table I lists the results of 26 determinations of pool size and turnover rate. Additional experiments were performed but the results were not considered to be acceptable technically. The values in the column entitled "Maximum Days for Equilibration" refer to the length of time before the logarithmic curves become straight. The immediate pool has been calculated according to the considerations already discussed. If more than one initial point deviated from the straight line, a second logarithmic curve was used in order to determine the y intercept of the original curve.

TABLE I

Uric acid pool size and turnover rate in various normal and pathological subjects

Experiment Number	Patient	Diagnosis	Clinical Treatment	Maximum Days for Equilibration	Immediate Pool (mgm. Uric Acid)	Apparent Pool (mg Uric Acid)	Turnover Rate (Pools/day)
3-1	S.B.	Gout		0.61	3031	3444	0.504
4-1	S.B.	Gout	Colchicine	0.87	1909	3389	0.350
6-1	W.S.	Gout			3430		0.477
6-2	W S.	Gout	Colchicine		3175		0.495
6-4	W.S.	Gout	Cortisone		3667		0.497
7-2	I.M.	Gout	Colchicine	0.63	2894	3437	0.960
10-1	G.D.	Gout		1.14	2104	2931	0.394
13-1	M.B.	Gout		0.37	1952		0.421
13-2	M.B.	Gout	ACTH	0.39	2139		0.484
17-1	W.de R.	Leukemia		0.35	5489		0.398
17-2	W.de R.	Leukemia	ACTH		6639		0.484
12-5	J.H.T.	Hyperur.		0.56	874	1772	0.642
12-6	J.H.T.	Hyperur.	ACTH	0.58	968	1512	0.705
21-1	E.S.	Hyperur.		1.37	1288	2114	0.544
19-1	W.G.	Normal		0.18	1019		0.785
19-2	W.G.	Normal		0.26	1019		0.822
9-1	C.C.	Rh. Arth.		0.38	1228		0.827
9-2	C.C.	Rh. Arth.	Colchicine	0.31	1173		0.620
9-3	C.C.	Rh. Arth.		0.37	1094		0.806
9-4	C.C.	Rh. Arth.	Cortisone		818		1.133
11-1	K.S.	Rh. Arth.		0.36	937		0.896
11-2	K.S.	Rh. Arth.	Colchicine	0.36	980		0.875
11-3	K.S.	Rh. Arth.		0.38	805		0.960
11-4	K.S.	Rh. Arth.	Cortisone	0.38	731		1.186
16-1	H.M.	Polycy.		0.38	1248		0.592
18-1	J.K.	Polycy.		0.27	1054	1766	0.675

The "apparent pool" is included in order to show the magnitude of error involved in merely extrapolating the straight portion of the curve. The turnover rate is presumed to be accurate so long as it is expressed as pools per day, but may be multiplied by the pool size only under certain circumstances as noted previously.

The several patients with gout and the one patient with chronic myelogenous leukemia were the only ones that showed a marked alteration of uric acid pool size and turnover rate. The other subjects which included normal persons without an elevated serum urate concentration, persons otherwise normal except for idiopathic hyperuricemia, patients with rheumatoid arthritis, and patients with polycythemia vera were alike in regard to their uric acid metabolism. For the control periods of all non-gouty, non-leukemic subjects, the mean pool size was 1,057 mg. with a standard deviation of 161. The mean turnover rate in pools per day was .755 with a standard deviation of .134. In contrast, no gouty subject had a metabolic pool as small as that of the non-gouty subjects. Furthermore, the turnover rate in the gouty subjects, with but one exception, did not exceed .510 pools per day.

The subject with chronic myelogenous leukemia had a high serum urate level so it was not unexpected that his pool size was elevated. The lowered turnover rate may accompany an increased pool size.

Two normal subjects with hyperuricemia [5] were included in this series. One subject, J. H. T., had had a concentration of serum uric acid above the normal for more than 16 years but had no familial history of gout. His metabolic pool size was somewhat below the mean as was also his turnover rate. The other hyperuricemic normal, E. S., had had no symptoms of arthritis, but had a brother with gout. The pool size of E. S. was the largest of all the subjects in the "normal" group and his turnover rate was nearly that of the gouty subjects. On the basis of these isolated observations it would appear that there are different types of hyperuricemia and that the hyperuricemia evidenced by relatives of gouty patients may well be a transition state between normal uric acid metabolism and the large pool size-low turnover

[5] A subject who consistently shows a serum uric acid concentration in excess of 6 mg. per 100 ml. by the colorimetric determination as performed in this laboratory is considered a hyperuricemic subject.

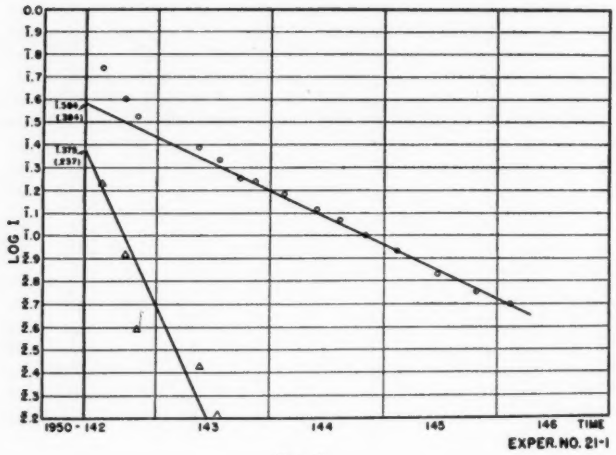

FIG. 2.

rate type of metabolism seen in patients with clinical gout. Whether the inability of the turnover rate to increase homeostatically with an increasing pool size might allow the pool size to increase progressively, or whether the lower turnover rate is a consequence of the enlarged pool size is a point for conjecture in the pathogenesis of gout.

Colchicine caused a dramatic decrease in the uric acid pool size, and a decrease in the turnover rate in one gouty subject, S. B. In another gouty subject the effect was equivocal. Colchicine administration was followed by a decrease in the uric acid turnover rate in two patients suffering from rheumatoid arthritis (C. C., K. S.). In one case the decrement was marked. Pool size was not greatly affected. Thus colchicine administration may lead to a decrease in the pool size of gouty subjects. Such a result would indicate a decrease in the rate of synthesis of uric acid either with or without a concomitant decrease in disposal rate such that the pool size would either remain constant or decrease. This is the first time that any definite action has been suggested for colchicine.

Cortisone was administered to three subjects. In one gouty subject, W. S., no metabolic effect was observed. In two rheumatoid arthritis subjects, however, the effect was marked especially on the turnover rate. In one patient, C. C., the turnover rate was increased from .806 to 1.133 and in the other patient, K. S., from .960 to 1.186. The pool size was also decreased in both instances. Such a coincidence of effects would suggest increased excretion (or catabolism) of uric acid with no reduction (or at least no corresponding increase) in the amount of uric acid entering the metabolic pool. It is known that ACTH increases the urinary excretion of uric acid (8) and cortisone might be suspected of acting similarly. Clinically it is known that cortisone is a more potent therapeutic agent in rheumatoid arthritis than in gout. Perhaps the metabolic effect noted in the arthritic subjects and not in the gouty is a reflection of this fundamental difference in effect.

ACTH was administered to one gouty, one leukemic, and one hyperuricemic subject. In each subject the pool size and the turnover rate increased. This suggests an increase in the rate of synthesis of uric acid without a comparable increase in the rate of excretion. These results are not inconsistent with the suggested uricosuric action of ACTH (8) but coincident with this, one must postulate an even greater effect on uric acid synthesis since only in this way can the pool size increase. Thus ACTH and cortisone would appear to differ in their effect since ACTH most probably has an accelerating effect on uric acid synthesis while cortisone need not have such an effect.

It should be remembered that all drugs used in these studies were given for limited periods only.

If the therapy had been continued for several weeks the findings might have been different. The phenomena noted above are what might be called primary or initial effects.

SUMMARY

The pool size and turnover rate of body uric acid were studied by the intravenous injection of N^{15} labeled uric acid into subjects before and after therapy with colchicine, cortisone, and ACTH. The subjects included normal and hyperuricemic subjects, as well as patients with gout, rheumatoid arthritis, polycythemia vera, and chronic myelogenous leukemia.

In some experiments it was observed that injected labeled uric acid did not immediately equilibrate with body uric acid. This was evidenced by the fact that when the logarithm of the isotope concentration of the urinary uric acid was plotted against time, the curve did not become straight for many hours. In such cases it was suggested that only the amount of body uric acid that immediately equilibrated with the injected uric acid ("immediate pool") could be accurately calculated. An extrapolation procedure was suggested for such cases.

Two hyperuricemic subjects, one having a gouty brother, and the other with no familial history of gout, were examined. The former, on the basis of uric acid pool size and turnover rate, appeared to represent a transition state between the normal and the gouty. The other subject was quite normal.

Colchicine in several patients led to a decrease in the uric acid turnover rate and in one gouty subject caused a concomitant decrease in pool size. It was suggested that colchicine might decrease the rate of synthesis of uric acid.

Both ACTH and cortisone increased the turnover rate but the pool size tended to increase following ACTH and tended to decrease following cortisone. A uricosuric effect could be postulated for both drugs but ACTH must have increased the synthetic rate of body uric acid. Cortisone showed its greatest effect in patients with rheumatoid arthritis. The effect in the one gouty subject was minimal.

Since all drugs were given for a limited period, the results would only reveal the primary effects of such drugs and would not suggest what might occur after prolonged administration.

PROTOCOL

W. G., age 26, male. Has no past history of clinical significance. A normal concentration of serum urate was noted on several occasions. He was considered to be a normal subject.

J. H. T., age 48, male. Has no past history of joint disease and no pertinent clinical history. The concentration of serum uric acid when observed from time to time has been above normal for more than 16 years. No family history of gout. Considered to be a normal control with hyperuricemia only.

E. S., age 23, male. One brother has gout. E. S. has had no symptoms suggestive of acute or chronic gouty arthritis. He has had an elevation of the serum uric acid when determined. He is considered to be a normal person with hyperuricemia and a positive family history for gout.

G. D., age 43, male. Has had acute attacks of gouty arthritis for more than 10 years. He has extensive tophaceous deposits and is classified as suffering from moderately severe acute and chronic gouty arthritis.

S. B., age 44, male, a victim of acute attacks of gouty arthritis for 23 years. There have been no peripheral tophi and there are minimal X-ray changes in the joints suggestive of gouty arthritis. Has frequent attacks of acute gout and is considered to be a case of moderately severe recurrent acute gouty arthritis with minimal evidence of chronic gouty arthritis.

W. S., age 51, male. Has had symptoms of acute gouty arthritis for 17 years. He has had several peripheral tophi and X-ray evidence of osseous tophi. The diagnosis is intermittent acute gouty arthritis and chronic gouty arthritis, moderate.

M. B., age 50, male. Has had symptoms of acute gouty arthritis for 19 years. He has evidence of subcutaneous tophi and osseous tophi. The diagnosis is moderately severe acute and chronic gouty arthritis.

I. M., age 41, male. Has had symptoms of acute gouty arthritis for 10 years. There is no peripheral evidence of gout but X-rays show evidence of osseous tophi. He has mild chronic gouty arthritis.

C. C., age 26, male. Has had symptoms of rheumatoid arthritis for 14 years. There is evidence of extensive peripheral joint disease as well as rheumatoid spondylitis. He has a moderately severe crippling form of the disease.

K. S., age 36, male. Has had symptoms of rheumatoid arthritis for five years. There is evidence of moderate involvement of peripheral joints as well as moderate involvement of the spine. He has mild acute rheumatoid arthritis.

W. de R., age 35, male. A diagnosis of chronic myelogenous leukemia was made first in 1942. The patient had had many transfusions but none at the time these studies

were in progress. The white blood cell count was over 650,000 per mm.^{3} The red cell count was 2.7 million and the hemoglobin 7.5 gm. Serum uric acid was as high as 20 mg. per 100 ml. The patient died a few days after this study was terminated.

J. K., age 63, male. Patient had had symptoms related to polycythemia vera for more than two years. The red blood cell count was 7.8 million and the white blood cell count was 17,000. The oxygen capacity was 27.6 vols. per cent and the cell volume 75.2 per cent. The patient had received no specific treatment at the time these studies were completed.

H. M., age 73, female. Patient had had symptoms which were probably related to polycythemia vera for more than two years. The oxygen capacity was 26 vols. per cent. The red blood cell count was 9 million, the hemoglobin 17.9 gm., and the white blood cell count 27,000.

ACKNOWLEDGMENT

The authors wish to acknowledge the invaluable assistance of Donald Wilson and Mrs. Dorothea Tamborski as well as the advice and assistance of Frank S. Stein and Ernest Lehmann, members of the Physics Department of the University of Buffalo.

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THE EFFECT OF BENEMID (P-[DI-N-PROPYLSULFAMYL]-BENZOIC ACID) ON URIC ACID METABOLISM IN ONE NORMAL AND ONE GOUTY SUBJECT [1, 2]

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Benzoic acid derivatives such as carinamide (4-carboxy-phenyl-methane-sulfonanilide) have been reported to be effective uricosuric agents, presumably by blocking renal tubular resorptions of uric acid. Benemid (p-[di-n-propylsulfamyl]-benzoic acid), another compound of this type, has recently become available. In patients with tuberculosis undergoing treatment with PAS (p-aminosalicylic acid) the simultaneous administration of Benemid tended to prevent the rapid renal excretion of the PAS. Since this effect was presumably due to some blocking of renal tubular activity, it became of interest to test the effect of Benemid on uric acid excretion.

The present study was carried out on two subjects. One was an idiopathic hyperuricemic subject (J. H. T.) who appeared from previous studies to have a normal uric acid metabolism; the other subject (W. S.) was known to be suffering from gouty arthritis and had been studied previously in this laboratory.

Before and after a course of Benemid therapy, N^{15} labeled uric acid was injected intravenously for the determination of uric acid pool size and turnover rate. Uric acid excretion was followed quantitatively by the isotope dilution method.

EXPERIMENTAL PROCEDURES

Each subject was injected intravenously with N^{15} labeled uric acid (25–50 mg, 16 atom per cent excess) and urine samples were collected for several days. The subject then began the daily ingestion of 2 gm of Benemid. On the third day of this regimen, labeled uric acid was again injected. Benemid ingestion was continued until the end of the study.

The determination of uric acid pool size and turnover rate employed the same materials and techniques as previously reported (1). In addition, an aliquot of each urine sample or combination of samples was taken for quantitative uric acid determination by the isotope dilution method. To this aliquot were added 25–50 mg of purified N^{15} uric acid of approximately 1 atom per cent excess dissolved in dilute sodium hydroxide solution. The molecules of added uric acid were presumed to mix completely with the molecules of uric acid present in the aliquot. A representative sample of this mixture of uric acid molecules was precipitated and its isotope concentration compared with that of a sample of uric acid precipitated from the same urine without augmentation. The amount of uric acid present in the original urine was calculated by the isotope dilution principle.

From standard solutions of uric acid it was demonstrated that this method under conditions comparable to those used for urine, gave results accurate to within 2 per cent. This implied that for a daily excretion of 800 mg the determination would be accurate to within $\pm$ 16 mg. It was believed that such results were considerably better than could be expected from any of the usual methods for the quantitative determination of urinary uric acid. Furthermore, the material determined as uric acid was uric acid, not a mixture of uric acid and non-uric acid chromogen as in the usual colorimetric procedures.

Serum uric acid concentrations were determined by the method of Folin (2). Since these values were only used as a clinical guide and not as the basis of further calculation, it is not so necessary that they be highly specific for uric acid.

RESULTS

Figure 1 is a graph of the isotope concentration of the urinary uric acid of the non-gouty subject (J. H. T.) in the period before the ingestion of Benemid. On day 160, Benemid ingestion was begun and was continued through day 166. Figure 2 shows the isotope concentration after the injection of labeled uric acid on day 163 and during the period of Benemid ingestion. The uric acid pool size in the control period was calculated by assum-

[1] Part of the data from this paper may be submitted by Royden Rand to the Graduate School of the University of Buffalo in partial fulfillment of requirements for the degree of Doctor of Philosophy in the Department of Biochemistry.

[2] This work has been carried out under grants from the Weed Foundation, Buffalo, New York and the National Institutes of Health, Public Health Service.

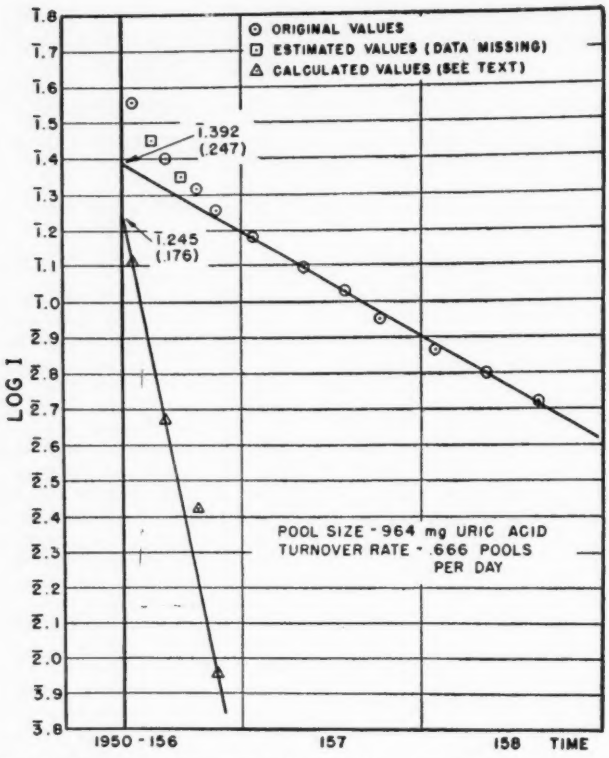

FIG. 1. ISOTOPE CONCENTRATION OF URINARY URIC ACID OF SUBJECT J. H. T. BEFORE BENEMID INGESTION

ing a second additive process according to the method previously outlined (1). This was unnecessary in the Benemid period. In the control period the amount of body uric acid with which the injected uric acid immediately equilibrated was calculated to be 964 mg. The turnover rate was .666 pools per day. After Benemid therapy the immediate pool size dropped to 466 mg, and the turnover rate rose to 2.457 pools per day. These results are consistent with the interpretation that Benemid is an effective tubular blocking agent and inhibits the resorption of uric acid. Under such circumstances some uric acid from the body pool should be excreted, leading to a decrease in the pool size and an increase in the turnover rate. (If the rate of synthesis of body uric is constant then a decrease in the pool size is equivalent to an increase in the turnover rate in pools per day.)

In Figure 3, the daily uric acid output of the same subject has been represented by a bar graph. On the same graph has been plotted the serum uric acid concentration. The fall in serum uric acid concentration and the rise in urinary uric acid excretion during Benemid therapy is apparent.

The mean daily uric acid excretion during an 11 day control period which included all days of urinary collection before and after Benemid administration was 830 mg. The "extra" uric acid excreted during the seven days on Benemid was 2705 mg. This was several times the magnitude of the 498 mg decrease in the immediate pool size after Benemid administration. At least two explanations might be offered for this. One is that this "extra" uric acid is not derived exclusively from the immediate pool but from a more remote pool of uric acid. At present there does not seem

to be any good way of testing this hypothesis. Another explanation is that with the renal tubules blocked, more exogenous uric acid finds its way into the urine. This hypothesis cannot be rigorously proved, but there are isolated facts that tend to support it. One is that subjects on Benemid continue to excrete "extra" uric acid as long as they continue taking the drug. Since the decrease in the serum uric acid concentration and the decrease in pool size occur within a day or two of the beginning of therapy, it would seem that the body would soon reach a state of minimum uric acid content and that thereafter "extra" uric acid would of necessity arise from external or dietary sources. Normally some uric acid does arise from dietary sources since it has been shown in this laboratory (3) that different dietaries, specifically high-fat as against high-protein diets, cause a significant difference in uric acid excretion.

The labeled uric acid that was injected into the non-gouty subject was not completely recovered as urinary uric acid. By day 162 no more N^{15} uric acid from the first injection was recoverable from the urine; hence it was assumed that all the injected labeled uric acid had already left the body pool. Since 28.5 per cent of the injected dose had

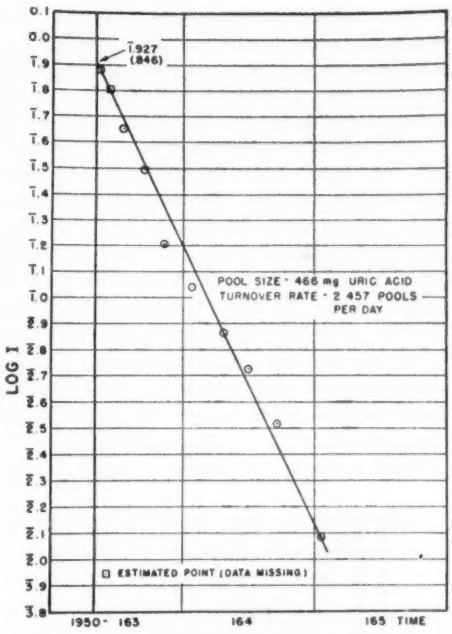

FIG. 2. ISOTOPE CONCENTRATION OF URINARY URIC ACID OF SUBJECT J. H. T. DURING BENEMID INGESTION

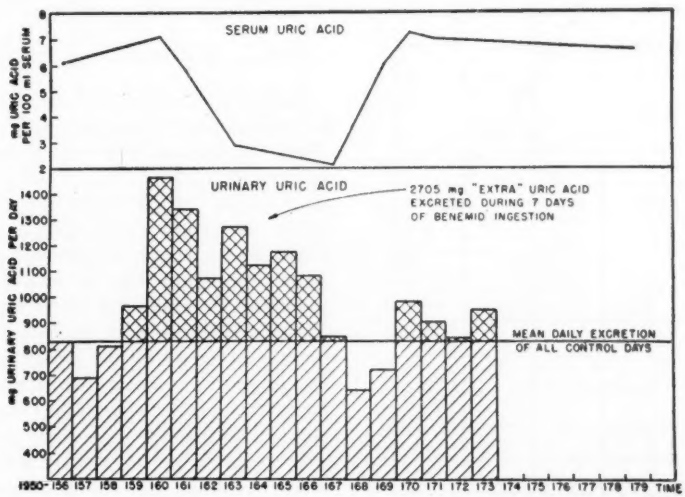
SERUM URIC ACID
mg URIC ACID PER 100 ml SERUM
URINARY URIC ACID
2705 mg "EXTRA" URIC ACID EXCRETED DURING 7 DAYS OF BENEMID INGESTION
MEAN DAILY EXCRETION OF ALL CONTROL DAYS
mg URINARY URIC ACID PER DAY
1950- 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 TIME

FIG. 3. SERUM URIC ACID CONCENTRATION AND URINARY URIC ACID EXCRETION OF SUBJECT J. H. T. BEFORE, DURING, AND AFTER BENEMID INGESTION

not been accounted for as urinary uric acid, it was assumed that this amount had been disposed of by another route, probably catabolism since there has been scattered evidence for such a process (4, 5).

Before and during the second N^{15} uric acid injection period the subject had been taking Benemid. The injected uric acid was excreted quickly, and 89.6 per cent was recovered as urinary uric acid. This is consistent with the hypothesis that one or more alternate mechanisms is competing for the uric acid that would otherwise be excreted by the kidney. If the N^{15} concentration of the body uric acid pool is quickly reduced, the amount of the injected uric acid that can escape by the alternate routes will be reduced. Benemid does cause such a rapid decrease in the N^{15} uric acid concentration of the body pool, presumably by blocking resorption of urinary uric acid.

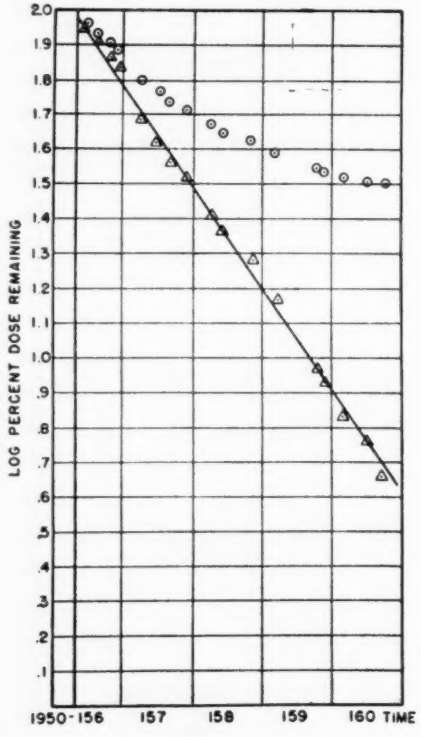

FIG. 4.
Subject J. H. T.—See text for explanation.

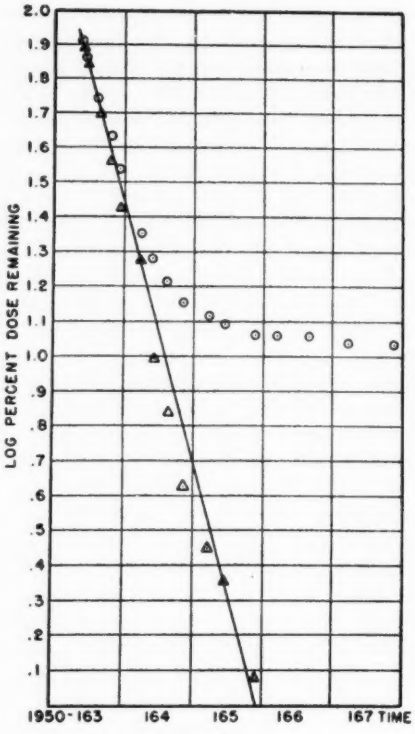

FIG. 5.
Subject J. H. T.—See text for explanation.

Further evidence for more than one pathway of uric acid disposal is presented in Figures 4 and 5. When the logarithm of the percentage of the dose apparently remaining in the body was plotted against time, the curve was not a straight line, neither during the control period (upper curve, Figure 4) nor during the Benemid period (upper curve, Figure 5). On the assumption that the amount of N^{15} uric acid remaining in the body pool should be calculated on the basis of only the uric acid excreted by the kidney, the data were recalculated. The amount of uric acid unaccounted for at the end of the study was subtracted from each datum and the logarithms of these values were again plotted in Figures 4 and 5 (lower curves). These new curves are straight lines. Thus the rate of urinary excretion of the labeled uric acid was directly related to the amount of the labeled uric acid actually left, not to the amount

merely unaccounted for on the basis of urinary excretion.

The second subject of this study, W. S., although suffering from gouty arthritis, responded to Benemid therapy in much the same way as the first subject. Benemid ingestion was begun on day 181 and continued through day 186. As can be seen from Figure 6, the uric acid pool size in his control period was 2205 mg and the turnover rate was .484 pools per day. After Benemid therapy (Figure 7) his pool size fell to 1622 mg and the turnover rate rose to 1.006 pools per day. This paralleled a marked drop in serum uric acid concentration as shown in Figure 8. Yet the drop in the size of the immediate pool was only 573 mg while the "extra" uric acid excreted during the six days on Benemid was 4889 mg as can be seen from the same figure. It is quite possible, especially in the gouty subject, that some of this "extra" uric acid is derived from a pool much larger than the immediate pool. There is also the suspicion that at least part of the "extra" uric acid is derived from dietary sources.

One striking difference between the behavior of the non-gouty and gouty subjects was the amount of the injected dose of isotopic uric acid recovered as urinary uric acid. In the gouty subject this was 98.9 per cent as can be seen from Table I. This

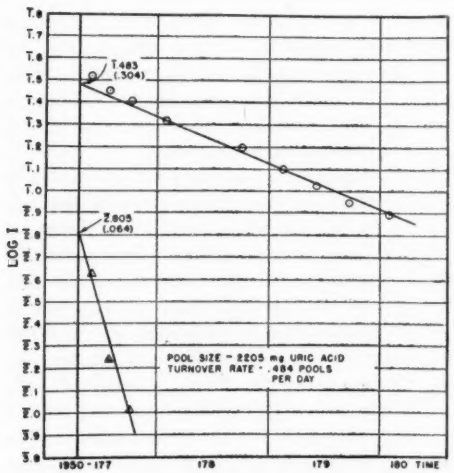

FIG. 6. ISOTOPE CONCENTRATION OF URINARY URIC ACID OF SUBJECT W. S. BEFORE BENEMID INGESTION

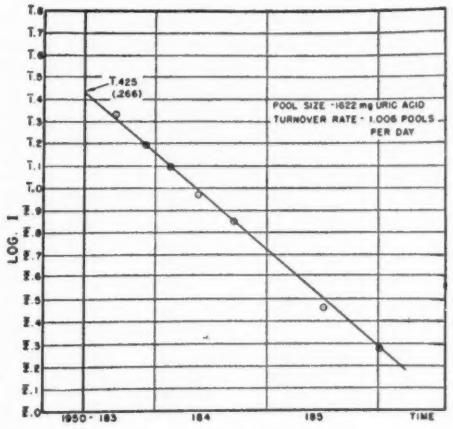

FIG. 7. ISOTOPE CONCENTRATION OF URINARY URIC ACID OF SUBJECT W. S. DURING BENEMID INGESTION

subject was still excreting a small amount of labeled uric acid when the study was terminated, so that the ultimate recovery might have been even higher.

Since this subject apparently did not catabolize any uric acid, it became feasible to calculate his pool size at various times. In this study the ini-

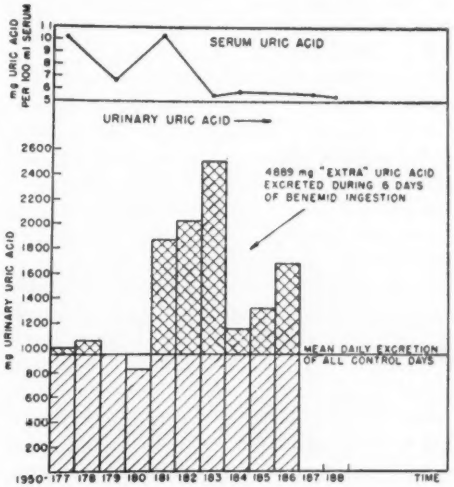
mg URIC ACID PER 100 ml SERUM
SERUM URIC ACID
URINARY URIC ACID
mg URINARY URIC ACID
4889 mg "EXTRA" URIC ACID
EXCRETED DURING 6 DAYS
OF BENEMID INGESTION
MEAN DAILY EXCRETION
OF ALL CONTROL DAYS
1950 177 178 179 180 181 182 183 184 185 186 187 188
TIME

FIG. 8. SERUM URIC ACID CONCENTRATION AND URINARY URIC ACID EXCRETION OF SUBJECT W. S. BEFORE AND DURING BENEMID INGESTION

TABLE I

Uric acid pool size in gouty subject at successive intervals both before and during Benemid therapy

Sample	Time at end of Sample	Mg x I	Amount Left Mg x I	%	Estimated I at end of Period	Pool Size (mg uric acid)
1*	177.312	0	831**	100.0		
2-3	528	71	760	91.5	.299	2540
4	649	74	686	82.6	.274	2500
5-6	917	53	633	76.2	.229	2760
7-8	178.271	55	578	69.5	.191	3020
9-10	531	61	517	62.3	.168	3080
11-13	179.003	73	444	53.4	.133	3340
14-15	281	21	423	50.9	.116	3640
16-17	545	28	395	47.5	.103	3840
18-20	913	36	359	43.2	.086	4180
21-22	180.267	21	338	40.7	.072	4700
23-26	948	36	302	36.4		
27-28	181.281	7	295	35.6		
29-35	182.229	62	233	28.0		
36-43	183.264	30	203	24.4		
44-45	503	233	386***	31.0	.236	1639
46-47	792	122	264	21.2	.177	1490
48-49	184.024	68	196	15.7	.140	1400
50	243	45	151	12.1	.112	1350
51-52	531	39	112	9.0	.084	1330
53-55	875	41	71	5.7	.059	1200
56-57	185.243	7	64	5.1	.041	1560
58-61	778	21	43	3.5	.024	1790
62-64	186.240	11	32	2.6	.016	2000
65-71	187.240	18	14	1.1	.008	1750

* Sample collected immediately prior to initial injection of isotopic uric acid

** Initial injection: 50 mg x 16.618 atom percent excess = 831

*** Second injection: 25 mg x 16.618 atom percent excess = 416
416 + 203 (remaining from first injection) = 619

tial dose minus the N^{15} uric acid excreted must equal the amount of N^{15} uric acid remaining in the body. These calculations were carried out in arbitrary units, since this simplified the calculations. The product of the number of milligrams of uric acid excreted during the first period and its isotope concentration in atoms per cent excess was subtracted from the product of the number of milligrams of uric acid in the injected dose and its isotope concentration. This new value, when divided by the estimated isotope concentration of the body pool at the end of this first period equalled the pool size in milligrams (neglecting the amount of uric acid contributed by the injected dose). The amount of isotopic uric acid excreted in the second period was similarly calculated and subtracted from the amount left at the end of the first period. This quantity divided by the estimated isotope concentration at the end of the second period gave the pool size at the end of the second period. As is apparent from Table I, the pool size increased constantly up to the point where it was no longer possible to make a satisfactory estimate of the isotope concentration of the body pool. When the pool size was plotted against time no asymptotic tendencies were evident so that an ultimate pool size could not be calculated. This may be due to the fact that in the body there are relatively large amounts of slowly equilibrating uric acid, perhaps in a solid phase.

In the Benemid period, the pool size started at a value closely approximating the pool size calculated by the graphical method and fluctuated down and up. This did not necessarily mean that there were not more than 1,600 mg of uric acid in the whole body at this time. It may be that no appreciable amounts of labeled uric acid had an opportunity to equilibrate with the less accessible depots (tophi, bound phases) of body uric acid because of the rapidity with which the N^{15} uric acid was excreted by the blocked tubules. The tendency of the uric acid pool size to increase toward the end of this study was not immediately explainable, but the last two or three values should

not be emphasized because the error of the isotope determination in this region is difficult to assess.

SUMMARY

One normal and one gouty subject have been injected intravenously with N^{15} labeled uric acid. The isotope concentration of the urinary uric acid was determined and the amount of urinary uric acid excreted was determined by the isotope dilution method. After a suitable control period each subject received oral daily doses of 2 gm of Benemid (p-[di-n-propylsulfamyl]-benzoic acid), a potent uricosuric agent, and subsequently were reinjected with N^{15} labeled uric acid.

In the normal and the gouty subjects the amount of body uric acid that would immediately equilibrate with injected N^{15} uric acid decreased markedly after Benemid therapy, and the turnover rate of the body uric acid pool increased greatly.

The amount of "extra" uric acid that was excreted during the period of Benemid therapy was much greater than the amount that disappeared from the immediate pool. Some of this "extra" uric acid, especially in the gouty subject, might have come from a more remote depot of uric acid but other evidence suggests that the diet, under the conditions of this experiment, may have contributed to this "extra" uric acid.

In the control period of the non-gouty subject only 71.5 per cent of the injected N^{15} uric acid was recovered as urinary uric acid up to the time when its excretion ceased. During Benemid therapy the recovery of a second dose of N^{15} uric acid was 89.6 per cent. This was explained as being due to the more rapid excretion of the N^{15} uric acid in the second period, thus decreasing the time during which the labeled uric acid could be disposed of by a competing catabolic or other disposal route. The recovery of injected N^{15} uric acid in the total of both periods of the gouty subject was 98.9 per cent up to the time when excretion of the isotope had virtually ceased. This gouty subject apparently did not catabolize uric acid or dispose of it in any other way than by urinary excretion.

Since in the gouty subject all of the injected labeled uric acid was excreted as urinary uric acid, it was possible to calculate the uric acid pool size at succeeding time periods. The size of the pool in the control period of this subject rose from approximately 2,700 mg at the time of injection of the labeled uric acid to 4,700 mg within three days. It could not be reliably estimated thereafter. After Benemid therapy the pool size fluctuated around 1,600 mg.

Benemid was shown, both in the gouty and non-gouty subject, to be a potent uricosuric agent, this action being associated with a reduction in the serum urate concentration and a reduction in the pool size of body uric acid.

ACKNOWLEDGMENT

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